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A U S T R A L I A

Functional Neuroimaging of Placebo Inhibition in Capsaicin Induced Urge-to-Cough

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Abstract

Background

An abnormal or persistent urge-to-cough is a common symptom of respiratory disease with few effective treatments. Evidence suggests that both cough and the urge-to-cough are particularly susceptible to placebo effects, indicating inhibitory processes in the central nervous system that are capable of reducing the urge-to-cough. Little is known about how these circuits are organised in the human brain. Knowledge of the neural basis of placebo inhibition of urge-to-cough may help to develop targets for future antitussive therapies. These studies investigate the effect of placebo on urge-to-cough using behavioural studies and functional magnetic resonance imaging (fMRI).

Aims:

- 1) To determine whether placebo conditioning can modulate the perception of capsaicin-evoked urge-to-cough in human subjects.
- 2) Using fMRI, to establish whether placebo induced reduction of the urge-to-cough causes a commensurate reduction in activity in cortical brain regions involved in sensing capsaicin-evoked urge-to-cough, and to identify regions that may be involved in producing this effect.
- 3) Using fMRI optimised for the brainstem, to establish if placebo induced reduction of the urge-to-cough also causes inhibition in subcortical regions involved in processing signals from the airways.

Methods

Three independent experiments were designed in which healthy adult volunteers underwent a series of inhalations of a substance that causes an urge-to-cough (capsaicin, the active ingredient in hot chilli peppers), preceded by either placebo treatment (via a placebo inert gas inhaler or normal air via a nasal cannula), which participants believed was a local anaesthetic, or no treatment. Participants rated their urge-to-cough following each capsaicin inhalation period. Prior to the sessions participants were conditioned to believe the treatment would be effective by surreptitiously lowering the dose of capsaicin following placebo administration. Three separate cohorts completed the protocol. The first experiment (Chapter 3) optimised a protocol to test whether the psychophysical aspects of capsaicin-evoked urge-to-cough were modifiable by placebo

intervention. The second experiment (Chapter 4) then employed this protocol along with blood-oxygen-level dependent (BOLD) fMRI to detect changes in cortical activation that accompanied the placebo response, while the third experiment (Chapter 5) used BOLD fMRI optimised for assessing subcortical and brainstem responses.

Results

Placebo administration resulted in a significant (up to 40 percent) reduction in participants' ratings of urge-to-cough. fMRI showed capsaicin-evoked activations in a network of regions including primary motor and sensory cortices, supplementary motor area, insula, mid cingulate cortex and orbitofrontal cortex. Placebo administration significantly reduced BOLD signal responses following placebo treatment in a distributed network of cortical regions that are normally activated during capsaicin inhalation, including primary and secondary somatosensory cortex, primary motor cortex, mid cingulate cortex and supplementary motor area. Regions in the dorsolateral prefrontal and posterior parietal cortices that have been implicated in producing placebo analgesia also showed increased activation following placebo treatment. In the dorsolateral prefrontal cortex the magnitude of the activation was significantly correlated with the magnitude of placebo effect in individuals. This reduction in capsaicin-evoked activation following placebo was also observed in the pons and rostral medulla in the brainstem.

Conclusions

These studies are the first to confirm that administration of a placebo can alter perception of experimentally-evoked urge-to-cough. They also provide further evidence that higher brain networks are important in modulating responses to airway irritation. Consistent with subjective reports, placebo administration decreases capsaicin-related activation in brain regions that process incoming sensory signals from the airways. This indicates that placebo treatment is capable of activating endogenous inhibitory networks in the central nervous system that can suppress the sensation of airway irritation. This process is likely to be mediated by higher cortical regions, such as dorsolateral prefrontal cortex, that can suppress processing of incoming sensory signals from the airways by descending inhibition of brainstem respiratory centres.

Declaration by author

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

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Publications during candidature

Peer Reviewed Papers

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Contributions by others to the thesis

Dr Farrell provided significant contributions to the design of the project and assistance with fMRI analysis and interpretation of data. Dr Mazzone contributed significantly to the conception of the project and provided assistance with interpretation of data and preparation of manuscripts. Dr Leonie Cole provided assistance with experimental design. Ms Ayaka Ando and Ms Camille Shanahan provided assistance with data collection. Dr Michael Kean contributed technical expertise related to fMRI data acquisition.

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List of Abbreviations

ACC	Anterior Cingulate Cortex
ANOVA	Analysis of Variance
BA	Brodmann Area
BBR	Boundary Based Registration
BOLD	Blood Oxygenation Level Dependant
C2	Lowest dose of capsaicin to elicit 2 or more coughs
C5	Lowest dose of capsaicin to elicit 5 or more coughs
CBF	Cerebral Blood Flow
CNS	Central Nervous System
COPD	Chronic Obstructive Pulmonary Disease
Cu	Lowest dose of capsaicin to elicit an urge-to-cough
deHgb	Deoxygenated Haemoglobin
DLPFC	Dorsolateral Prefrontal Cortex
EPI	Echo-Planar Imaging
fMRI	Functional Magnetic Resonance Imaging
FOV	Field of View
FWE	Family Wise Error
Hgb	Haemoglobin
HRF	Haemodynamic Response Function
INS	Insula
IPL	Inferior Parietal Lobe
MEG	Magnetoencephalography
MFG	Middle Frontal Gyrus
MI	Primary Motor Cortex
MNI152	Montreal Neuroscience Institute standard brain template
MPFC	Medial Prefrontal Cortex
MRI	Magnetic Resonance Imaging
nTS	Nucleus of the Solitary Tract (Nucleus Tractus Solitarius)
OFC	Orbitofrontal Cortex
PAG	Periaqueductal Grey
PET	Positron Emission Tomography
PFC	Prefrontal Cortex
PgACC	Pregenual Anterior Cingulate Cortex

PPC	Posterior Parietal Cortex
PreC	Precentral Gyrus
rACC	Rostral Anterior Cingulate Cortex
rCBF	Regional Cerebral Blood Flow
SI	Primary Sensory Cortex
SII	Secondary Sensory Cortex
SMA	Supplementary Motor Cortex
TE	Echo Time
TMS	Transcranial Magnetic Stimulation
TR	Repetition Time

Chapter 1 - Introduction

Cough

The cough reflex is a vital protective mechanism for clearing the airways of foreign matter such as aspirates, secretions, inhaled particulates, irritants or pathogens. A properly functioning cough reflex is essential to prevent airway damage and maintain normal airway function. There is however, much more to cough than the stereotypical motor response itself. Recent research has also begun to focus on the sensory aspects of cough, with the aim of understanding how this information is integrated in the central nervous system in both normal and pathological states.

In the simplest case, airway afferent neurons detect physical changes in the airways and this information is relayed to regions of the brainstem, initiating reflexive coughing. This motor act of coughing is a stereotypical expulsive reflex designed to clear material from the airways. It is classically defined based on three characteristic phases: an initial inspiration, followed by compression and expulsion. Given that the cough reflex is critical to respiration, this system is normally tightly regulated and can be observed even in decerebrate animals (Satoh, Shiba, Kobayashi, Nakajima, & Konno, 1998).

A number of factors however, differentiate cough from other respiratory reflexes. Cough can be not only reflexive, but also voluntary or behavioural. It is well known that, in humans, cough can be voluntarily inhibited or initiated under the right circumstances, such as consciously suppressing cough during a concert, or voluntarily coughing on demand. In addition, cough can be modified or suppressed by internal factors such as attention (Van den Bergh, Vranken, Dhooge, Silva, & Dupont, 2011) and anxiety (Davenport, Vovk, Duke, Bolser, & Robertson, 2009; Lavorini et al., 2010), or external factors such as social context (Van den Bergh, Van Diest, Dupont, & Davenport, 2012). This implies that in addition to a brainstem mediated reflex loop, there must also be descending input from the cortex. This level of voluntary control is unusual for a respiratory reflex, leading to the current interest in central nervous system mechanisms involved in generating and maintaining cough.

Despite advances in understanding the basic physiology of reflex cough, particularly from animal models, there remains a lack of knowledge of how the cough sensory system is organised in humans. Given the lack of effective antitussive (cough suppressant) treatments currently available, there is a pressing need for research in this area.

Clinical Conditions Involving Abnormal Cough

Complications often arise when the urge-to-cough becomes sensitised or desensitised, leading to constant unrelieved urge-to-cough, excessive reflex coughing or, in the case of desensitisation, a loss of awareness of airway function and ineffective coughing. Acute cough associated with viral or bacterial infection generally resolves itself with minimal complications. Chronic cough however has proved difficult to treat and therefore is often poorly managed, with severe consequences for patients' quality of life (French, Irwin, Curley, & Krikorian, 1998). Epidemiological studies estimate the rate of chronic cough (defined as a cough lasting more than 8 weeks) in developed countries at between 9 and 33% of the adult population (Chung & Pavord, 2008), while an abnormal or persistent cough is among the most common reason for seeking medical treatment (McGarvey & Morice, 2006), being the primary reason for over 30 million visits to primary care providers in the United States annually ("National Ambulatory Medical Care Survey: 2010 Summary Tables "). It is therefore surprising that so few effective antitussive treatments are available, and there is an increasing awareness of the need for new therapeutic tools (Bolser, 2006; Irwin et al., 2006).

Antitussive Medications

The most commonly prescribed antitussive treatments are codeine and dextromethorphan, both of which act on the central nervous system. A number of recent double blind, placebo-controlled studies have shown that codeine is not significantly more effective than placebo in suppressing cough due to upper airway disorders (Bolser & Davenport, 2007; Eccles, Morris, & Jawad, 1992). The efficacy of dextromethorphan has also been questioned following clinical trials showing no effect over placebo (Korppi, Laurikainen, Pietikäinen, & Silvasti, 1991; Paul et al., 2004; Taylor, Novack, Almquist, & Rogers, 1993) in treating cough due to upper respiratory tract infections, particularly in children. A meta-analysis of 25 randomised, placebo-controlled studies of various over the counter antitussives in adults and children concluded "there is no good evidence for or against the effectiveness of over the counter medicines in acute cough" (Smith, Schroeder, & Fahey, 2008).

Chronic Cough Disorders

Chronic cough and persistent urge-to-cough are associated with a variety of common medical conditions, among them asthma, bronchitis, chronic obstructive pulmonary disease and gastro-oesophageal reflux (Morice, 2008). Chronic cough can also be invoked by environmental factors, such as smoking or pollution, sensitisation following a respiratory infection or by drug treatment

(particularly angiotensin-converting enzyme inhibitors) (Dicpinigaitis, 2006). Conversely, an impaired cough reflex is a potentially severe symptom of neurological disorders such as Parkinson's disease (Ebihara et al., 2003; Fontana, Pantaleo, Lavorini, Benvenuti, & Gangemi, 1998), stroke (Horner & Massey, 1988; Ward et al., 2010), spinal cord or traumatic brain injury (Mansel & Norman, 1990; Wiercisiewski & McDeavitt, 1998) and other neuromuscular disorders (Hadjikoutis, Eccles, & Wiles, 2000; Mutluay, Gurses, & Saip, 2005; Szeinberg et al., 1988). Unlike acute infections, which are generally self-limiting, many of these conditions are life long and without specific treatments. Therapy therefore consists of symptom management, and chronic cough has proven to be a particularly difficult symptom to manage effectively (Dicpinigaitis, 2011; Morice & McGarvey, 2009).

Superficially, chronic coughing may appear to be a relatively innocuous complaint. However, coughing often causes secondary complications, including urinary incontinence, vomiting, rib fractures and loss of sleep. Perhaps most importantly from a clinical perspective, chronic cough leads to anxiety, social isolation and embarrassment, significantly impacting on quality of life (Chung & Pavord, 2008). Chronic cough patients have significantly higher rates of depression and anxiety than the general population (Dicpinigaitis, Tso, & Banauch, 2006; McGarvey et al., 2006), with reported rates of depressive symptoms comparable to those seen in patients with chronic heart failure and diabetes (Dicpinigaitis et al., 2006).

Idiopathic Cough

In a proportion of patients reporting chronic cough, the aetiology remains unexplained even after extensive clinical work-ups. It appears that in at least some of these patients a chronic unresolved cough may be due to an abnormal or overly sensitised cough reflex, rather than a direct result of an underlying condition (O'Neill, McMahon, & Udem, 2013; Pavord & Chung, 2008; Woodcock, Young, & Smith, 2010). It is now thought that neuronal plasticity may cause a remodelling of the cough reflex regulatory network following the initial infection or disease, analogous to the central sensitisation that is thought to occur in chronic neuropathic pain (O'Neill et al., 2013). This can lead to a heightened response to normally innocuous stimuli. However, as most of the current research into cough neurophysiology comes from animal models, the precise pathways and mechanisms that might be involved in this process in humans are still unexplained.

Over the counter treatments such as dextromethorphan are usually ineffective in this group of patients (Bolser, 2006), and habituation often occurs with long term administration of opioid based

cough suppressants, as well as the risk of side effects (Iyer & Lim, 2013). As traditional treatments tend to be ineffective in this disorder, it is hoped that knowledge of the central pathways involved in sensing and inhibiting the urge-to-cough may be of use firstly in understanding what causes cough reflex hypersensitivity, and secondly in identifying prospective therapeutic targets. It is possible that either pharmaceutical or behavioural modification to enhance or restore the function of inhibitory circuits could lead to more effective outcomes than currently available treatment options.

The Urge-to-Cough

The physical discomfort caused by clinical cough is not limited to the excessive motor act of coughing. Often the primary complaint is rather a constant sense of irritation that is not relived by physical coughing. Cough is generally preceded by this sensation of irritation, usually identified as a feeling of an itch or tickle in the back of the throat. This is defined as the urge-to-cough, preceding cough similar to the way in which a sensation of itch leads to a conscious drive to scratch.

Cough can either be generated reflexively, before a sensation of urge-to-cough is felt, or it can be produced via a conscious sensory process, where stimulation of the airways causes recognition of a desire to cough, depending on both stimulus intensity and context. In this case, the eventual production of cough relies on a complex chain of events, beginning with sensory input from the airways being passed to subcortical nuclei, before being relayed to the cortex where cognitive, emotional and contextual information is integrated. Descending pathways then feed back to brainstem regions that initiate or suppress the motor response.

Studying this sensory experience of urge-to-cough, rather than the cough reflex itself, is likely to be more useful in terms of understanding pathological cough disorders. Additionally, understanding how sensory signals from the airways are processed in the central nervous system may be a more productive research strategy for developing antitussive therapies, as disrupting the protective cough reflex is not a clinically desirable aim.

Modulation of the Urge-to-Cough

Like cough, the urge-to-cough is modifiable by both internal states and external influences. If we are to fully understand the central nervous system changes underlying cough disorders then knowledge of the sensory pathways that give rise to an urge-to-cough is an important first step.

The urge-to-cough can be modified by anxiety (Davenport et al., 2009), exercise (Lavorini et al., 2010) or upper airway respiratory infections (Dicpinigaitis, Bhat, Rhoton, Tibb, & Negassa, 2011). This strongly implies the existence of endogenous mechanisms for modulating the urge-to-cough, most likely involving inhibitory mediators in the central nervous system. If these inhibitory regions can be identified, the potential exists for them to be utilised as novel prospective targets for drug development.

Placebo Modulation of Urge-to-Cough

Reports of high rates of placebo responses in antitussive trials also support the supposition that urge-to-cough can be endogenously modulated. The placebo effect has been successfully used to manipulate the sensory experience of interoceptive states such as pain, and the possibility exists for its use as an investigative tool to better understand which brain regions are involved in modulation of the urge-to-cough.

There has been growing interest surrounding placebo effects since Beecher reported in 1955 that 35% of patients responded positively to placebo treatment (Beecher, 1955), leading to the widespread adoption of randomised placebo controlled clinical trials. Placebo effects have since been reported in numerous conditions, from pain to psychological disorders such as depression (Leuchter, Cook, Witte, Morgan, & Abrams, 2002; Walsh, Seidman, Sysko, & Gould, 2002) and neurological disorders such as Parkinson's disease (de la Fuente-Fernández et al., 2001; Lidstone, Schulzer, Dinelle, & et al., 2010). Clinically, debate is ongoing about whether attempts should be made to harness this effect for therapeutic benefit, or whether the use of deception is unethical (Brody, 1982; Miller & Colloca, 2009; Price, Finniss, & Benedetti, 2008).

Experimentally, the placebo effect has proven useful as a method of modifying the subjective experience of a stimulus, allowing for the study of inhibitory processes within the central nervous system. If placebo effects can be induced experimentally in a model of cough, this would then allow us to investigate the functional neuroanatomical regions involved in modifying the urge-to-cough.

Placebo Effects in Cough

It has been estimated that up to 85% of the treatment efficacy of over-the-counter anti tussive medications in clinical trials is due to placebo effect (Eccles, 2010). However, since these clinical trials do not routinely include a no treatment arm, it is unclear what factors contribute to this effect. Any observed response may be due to a variety of reasons, such as regression to the mean (the fact

that people tend to enrol in clinical trials when their symptoms are at their worst), natural resolution of symptoms, or the effect of substances other than the active pharmaceutical, all of which make up the perceived placebo effect. Regardless of the relative contributions of these factors, the magnitude and consistency of placebo effects reported in studies of antitussives is larger than that seen in studies of similar sensory processes such as placebo analgesia (Hróbjartsson & Gøtzsche, 2004a; Vase, Riley, & Price, 2002).

The large improvements in symptoms with placebo seen in clinical trials of new antitussives could be one of the reasons that so few new drugs have been approved for the treatment of cough. It has been speculated that these effects may mask any true responses, leading to premature rejection of antitussives that may eventually have proved clinically useful. Investigating this experimentally in healthy subjects allows us to control for these factors and estimate the magnitude to which the “true” placebo effect might contribute to these responses.

Placebo Effects in Pain

Placebo responses can be utilised as a method of manipulating the experience of a given stimuli. This has been most commonly studied in relation to analgesia, in order to investigate the functional roles played by various cortical regions involved in pain perception. This research has proved that placebo analgesic responses are not only due to response bias, as was previously speculated (Price et al., 2008). Sensory areas in the cortex show significantly lower levels of activation when a subject receives a placebo treatment prior to painful stimulation, indicating that subjects are not merely retrospectively labelling their pain levels as less intense (Wager et al., 2004). High resolution imaging of the spinal cord during a placebo analgesia paradigm has further shown that there is reduced neural activity during pain processing even at the level of spinal cord neurons (Eippert, Finsterbusch, Bingel, & Buchel, 2009b), indicating a potential mechanism of placebo analgesia whereby descending modulation could cause inhibition of incoming sensory signals.

Models of Placebo Effect

Whilst the precise neurobiological mechanisms of placebo are still speculative, much progress has been made towards defining precisely what the placebo effect is, and how the brain might orchestrate it. Placebo effects have been primarily studied in relation to nociception, or the perception of pain. Other sensory systems are equally likely to be affected, such as the perception of tactile stimuli (Fiorio et al., 2012).

From a traditional point of view, the placebo effect is considered to be a learning process mediated by a combination of expectation and classical conditioning. Expectation can be elicited by verbal reinforcement, for instance: “this treatment will reduce your urge to cough”. Conditioning, in experimental studies, consists of a period where the level of stimulus is lowered without the subject’s knowledge to produce an association between the placebo and the desired outcome. In a clinical setting this necessarily becomes much more complex, as factors such as attitude, anxiety, past experience of effective or ineffective therapies and the relationship between patient and practitioner all contribute towards expectation, and the past experiences of the patient can themselves be considered a form of conditioning (Voudouris, Peck, & Coleman, 1985). This may contribute to the difficulties seen in treating chronic cough patients, who have usually been exposed to numerous ineffective treatments and therefore may have low expectations of positive treatment outcomes.

Conditioning can also be pharmacological, where placebo replaces an active treatment after a period of time. This has proven effective with a range of treatments, where drug specific effects persist even when placebo is given. For example, after opioid treatment pain is reduced with placebo alone (Amanzio & Benedetti, 1999), while following treatment with an immunosuppressant, production of immunomodulatory cytokines is inhibited (Ober et al., 2012). After analgesic treatment with buprenorphine, placebo produces respiratory depression, mimicking the side effects of opioid treatment (Benedetti, Amanzio, Baldi, Casadio, & Maggi, 1999). Similarly, placebo treatment causes a naloxone-reversible reduction in heart rate (Pollo, Vighetti, Rainero, & Benedetti, 2003). Placebo effects can also be induced simply by observing another person responding positively to an analgesic (Colloca & Benedetti, 2009).

It is thus clear that the “placebo effect” is not a singular entity, but must rather be regarded as a set of processes particular to the system being studied. As yet, despite much speculation, the underlying mechanisms involved in placebo effects in relation to cough have not been studied experimentally.

Models of Urge-to-Cough

The afferent pathway leading to a sensation of urge-to-cough is initiated by peripheral nerve receptors in the respiratory tract, particularly in the larynx, trachea and bronchi. Subpopulations of sensory neurons may be activated by either mechanical or chemical stimulation. Mechanical cough receptors in the lining of the epithelia react to physical stimulation, such as particulate matter, while

chemical receptors can be activated by a range of substances, including capsaicin (Hansson, Wollmer, Dahlback, & Karlsson, 1992; Widdicombe, 1995).

These vagal afferent nerves project from the airways to the brainstem, where they appear to converge primarily in the nucleus of the solitary tract (nTS). The nTS is an important site for integration of visceral sensory information, receiving and integrating afferent input from the gastrointestinal system, lungs, heart and airways (Boscan, Pickering, & Paton, 2002; Broussard & Altschuler, 2000; Kubin, Alheid, Zuperku, & McCrimmon, 2006). Central projections both inward and outward from the nTS are likely to play a role in modifying the urge-to-cough sensation. The nTS has projections to other brainstem nuclei including the reticular formation in the medulla and the parabrachial complex, particularly the Kolliker-Fuse nucleus (Torvik, 1956), as well as subcortical projections to the hypothalamus, thalamus and amygdala (Canning, 2007; Mazzone & Canning, 2002; Ohi, Yamazaki, Takeda, & Haji, 2005). As well as serving as the primary relay nucleus, there is evidence that some degree of integration of peripheral cough sensory information may also occur at the level of the nTS (Bolser & Davenport, 2002; Ohi et al., 2005).

Although tracing studies in animal models (primarily guinea pigs), have been invaluable in discovering the projections of subpopulations of sensory neurons arising from the airways, it is unclear to what extent these results translate to humans. Interest has thus turned towards non-invasive tools such as functional magnetic resonance imaging (fMRI) in order to establish whether the same pathways exist in humans.

Cortical Modulation of Urge-to-Cough

Cough is generally initiated following the sensation of an urge to cough. Rather than being a simple brainstem mediated reflex, as was considered the case until relatively recently, it is now apparent that the process of sensing and acting upon an urge-to-cough is considerably more complex and involves a network of cortical regions.

Evidence for this comes from multiple sources, including fMRI studies of cough and urge-to-cough (Mazzone, Cole, Ando, Egan, & Farrell, 2011; Mazzone, McLennan, McGovern, Egan, & Farrell, 2007), the effect of stroke on reflex cough (Addington, Stephens, Widdicombe, & Rekab, 2005) and the ability in humans to voluntarily produce or suppress cough.

The model developed by Eccles (Lee, Cotterill-Jones, & Eccles, 2002) proposes that brainstem respiratory centres are modulated by input from the cortex. We have previously shown that evoked cough activates cortical as well as subcortical regions (Mazzone et al., 2013). It therefore seems reasonable to hypothesise that voluntary suppression of cough involves downregulation of respiratory centres in the pons and medulla via top down cortical inhibition.

Functional Magnetic Resonance Imaging

Functional or blood oxygenation level dependant (BOLD) MRI uses the differing magnetic properties of oxygenated and de-oxygenated haemoglobin in the blood to provide an endogenous contrast mechanism without the need for exposing subjects to ionising radiation. Because the ratio of oxygenated to de-oxygenated haemoglobin is closely coupled to neuronal activation (Logothetis & Pfeuffer, 2004), this allows us to localise functional activity in healthy awake human volunteers by statistically comparing signal intensity when the subject is engaged in a particular activity to either rest or a control task.

The use of fMRI has enabled researchers to design studies which were not previously possible using either animal models or behavioural studies. For instance, human volunteers can report subjective experiences such as urge-to-cough. Recent studies using fMRI have shown that the network of cough related cortical areas is more extensive than was previously thought, incorporating inhibitory and regulatory regions (Mazzone, Cole, Ando, & Farrell, 2010). Increased knowledge of the basic mechanisms of cough reflex regulation at both a brainstem and cortical level is needed in order to better understand how dysfunction occurs in chronic cough disorders.

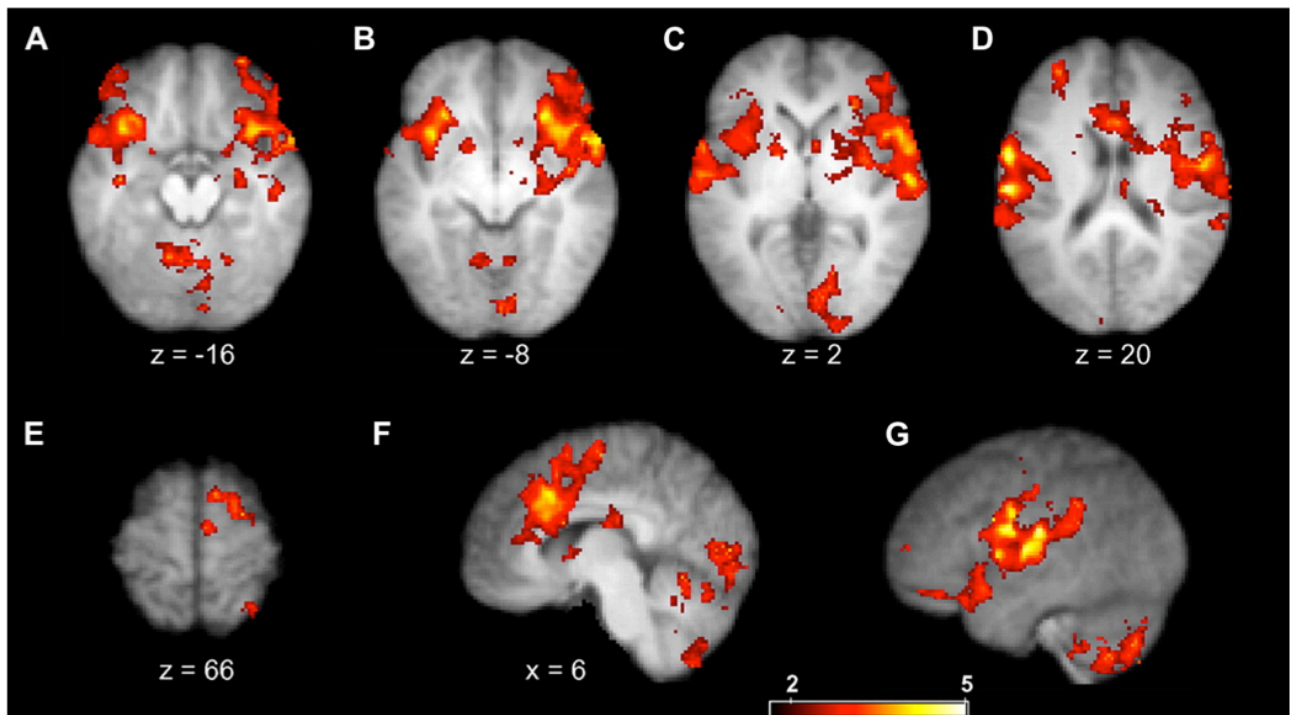
Functional Neuroanatomy of Cough and Urge-to-Cough

Previous studies by our group utilising fMRI during induced cough and urge-to-cough have shown increased activation in a network of cortical and sub-cortical regions (Figure 1) (Mazzone et al., 2011; Mazzone et al., 2007). These brain regions are likely to underlie the diverse processes involved in sensing and reacting to tussive stimuli, such as perception of intensity, localisation of irritation and motivation to initiate or suppress cough. By manipulating aspects of the sensory experience during fMRI, we have begun to ascribe functional roles to various components of the network.

We first identified a distributed network of somatosensory, premotor, limbic and paralimbic areas that were activated during capsaicin induced airway irritation (Mazzone et al., 2007)(see Figure 1).

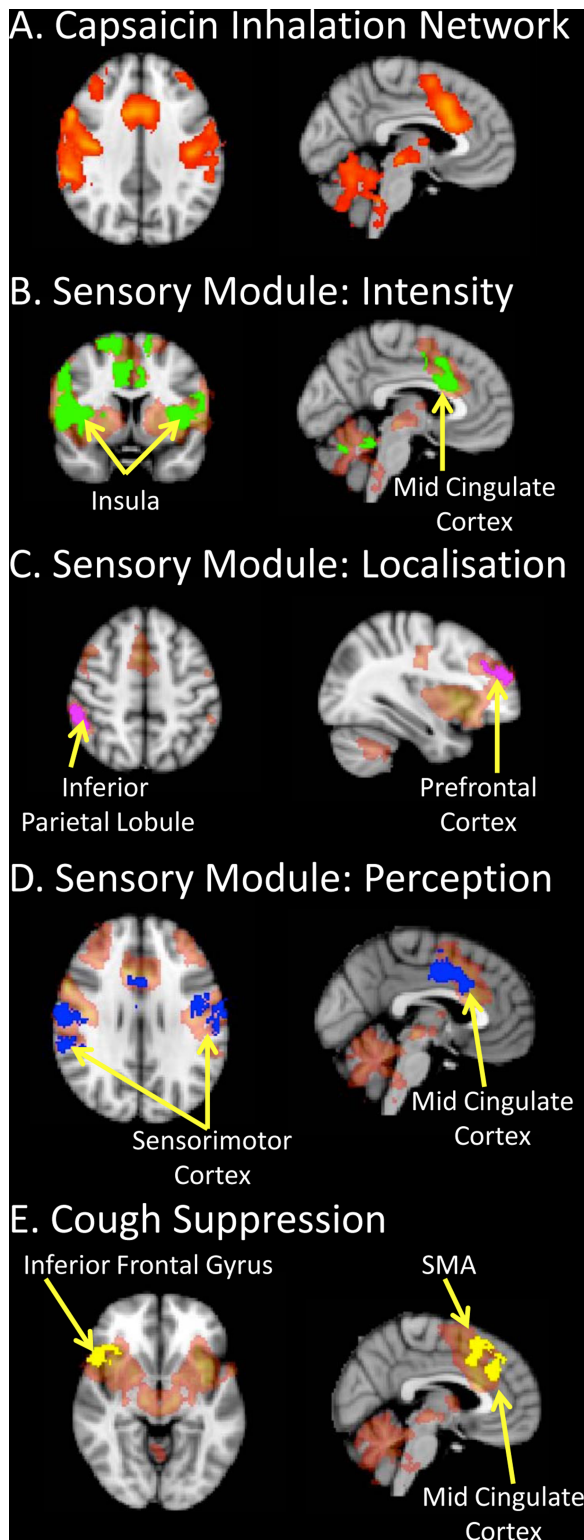
This network is likely to be composed of regions involved in sensing and acting upon information ascending from airway cough receptors, as well as cognitive and emotional processing of stimuli. Further research has focused on functional subdivision of this network. By modelling the variance in subjective ratings compared to the variance in stimulus intensity, it was shown that discrete sections of this network responded in either a stimulus-dependant or ratings dependant fashion, implying that they may be involved in different aspects of sensory processing (Figure 2) (Farrell, Cole, Chiapoco, Egan, & Mazzone, 2012).

Figure 1 - Activations associated with inhalation of capsaicin



The majority of regional activations were distributed in both hemispheres, including the orbitofrontal cortex (A), inferior frontal gyrus (A, B), anterior insula (B, C), superior temporal gyrus (C), and primary motor and somatosensory cortices (D, G). Midline capsaicin activations included the supplementary motor area (E) and the anterior midcingulate cortex (F). The locations of capsaicin activations in the primary motor and somatosensory cortices were at the caudal end of the central sulci in both hemispheres (D), and are clearly discernable on the three-dimensional rendering of the left hemisphere. Slice coordinates are according to the Montreal Neurological Institute (MNI) 152 brain and activations are rendered on the average of subjects' anatomical images after registration to the MNI brain. Reprinted with permission from (Mazzone et al., 2007).

Figure 2 - Functional Brain Maps of Sensorimotor Activations Following Capsaicin Inhalation in Humans



(A) Capsaicin inhalation is associated with the activation of a distributed network in the brain (Mazzone et al., 2007). We propose that this network is composed of several sub-circuits (modules) involved in sensory discrimination and motor control (panels B-E). Our published data indicate that discrete regional responses incorporate modules that (B) encode stimulus intensity, (C) identify stimulus location, (D) determine perceptual experiences and (E) can suppress evoked motor responses; see (Farrell et al., 2012; Mazzone et al., 2011). The module specific activations shown in green, pink, blue and yellow on panels B-E are superimposed on the distributed capsaicin inhalation network (shaded orange) as highlighted in panel A. See cited references for full details of experimental design, data analysis and interpretation. Reprinted with permission from (Mazzone et al., 2013).

Functional Neuroanatomy of Placebo Effect

Most research thus far has focused on placebo effects in relation to pain and analgesia, as this is a relatively simple system to study experimentally. Prior to the advent of neuroimaging techniques, placebo effects were often explained as a result of ratings bias, where participants either retrospectively labelled their experience as less intense, or subconsciously adjusted their ratings in line with the expectations of researchers (Hróbjartsson & Gøtzsche, 2001; Hróbjartsson, Kaptchuk, & Miller, 2011).

The first evidence for placebo as a process with a distinct neurobiological basis rather than as a purely psychological process came from the finding that placebo analgesia could be blocked by naloxone, an opioid antagonist, indicating that endogenous opioids were involved in producing placebo effects in pain (Levine, Gordon, & Fields, 1978). It has since been shown that while placebo responses following both expectation and conditioning with morphine were completely blocked by naloxone, conditioning using a non-opioid analgesic was not (Guo, Wang, & Luo, 2010). It is now accepted that both opioidergic and dopaminergic pathways are involved in the placebo analgesia response (Scott et al., 2008). Whether this is true of placebo effects in other sensory systems is not yet known.

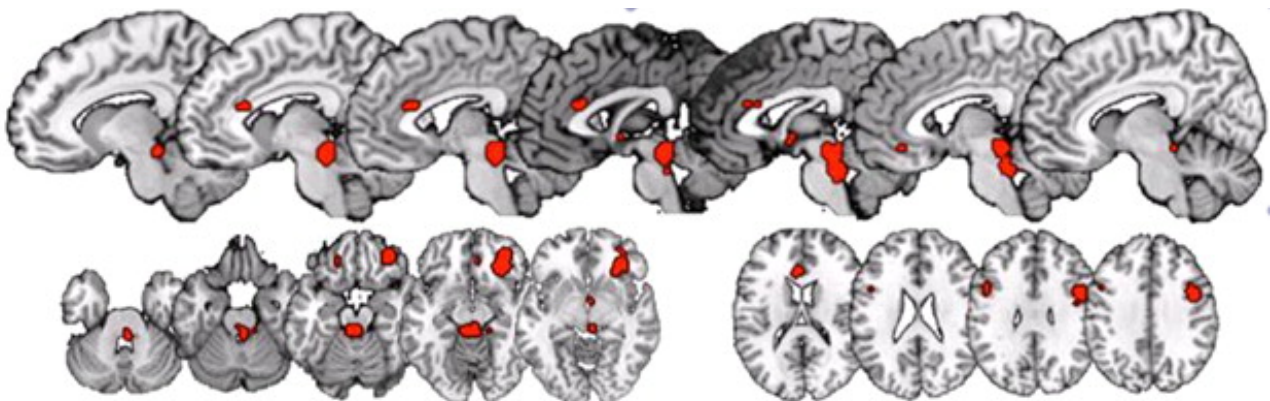
Positron emission tomography (PET) studies of placebo effects in analgesia enabled localisation of brain regions involved in the response to placebo, including those specific to various ligands. Wager et al showed that during heat pain following either placebo analgesia or no treatment, regional μ -opioid receptor binding was enhanced in the placebo condition in a number of key brain regions including dorsolateral prefrontal cortex (DLPFC), anterior insula, rostral anterior cingulate cortex (rACC), pregenual anterior cingulate cortex (pgACC), periaqueductal grey (PAG), amygdala, thalamus, and orbitofrontal cortex (Wager, Scott, & Zubieta, 2007). A similar study using a sustained pain protocol showed increased μ -opioid receptor binding in DLPFC (Brodmann Area 8 and 9), PgACC (BA24 and 25), anterior insular cortex and nucleus accumbens (Zubieta et al., 2005). Additionally, μ -opioid receptor activity in the right DLPFC was negatively correlated with the expectation of analgesia.

Further PET studies showed increased regional cerebral blood flow (rCBF) in medial prefrontal cortex (MPFC)(BA9), posterior parietal cortex (PPC)(BA7) and inferior parietal lobe (IPL)(BA40) after placebo administration in placebo responders, but not in placebo non-responders (Nemoto, Nemoto, Toda, Mikuni, & Fukuyama, 2007).

fMRI studies of placebo analgesia have also reported decreased activation following placebo in cortical regions associated with pain modulation. During painful stimuli following placebo, participants reported less pain as well as showing decreased activation in insula, thalamus and rACC (Wager et al., 2004). The decreases in behavioural pain ratings were significantly correlated with activity in DLPFC, OFC and rACC. fMRI studies have also consistently reported increased brain activation associated with placebo in regions including the lateral prefrontal cortex and OFC (Wager & Fields, 2013; Wager et al., 2004) (see Figure 3).

The DLPFC and OFC appear to play particularly important roles in the modulation of placebo effects during pain. DLPFC and OFC activation is correlated with midbrain activation in the vicinity of PAG (Wager et al., 2004), leading to speculation that placebo may be caused by top-down inhibitory processes originating in the prefrontal cortex. In support of this theory, placebo analgesia is abolished when transcranial magnetic stimulation (TMS) is used to transiently disrupt DLPFC functioning (Krummenacher, Candia, Folkers, Schedlowski, & Schonbachler, 2010).

Figure 3 - Regions Consistently Showing Greater Activation in fMRI Placebo Analgesia Studies



Adapted from (Meissner et al., 2011a). Results of placebo analgesia fMRI studies from 2004 to 2011 showing areas reported in three or more studies to show placebo related increases in activation, or placebo related correlations with brain activity, during administration or anticipation of pain.

Summary

Placebo seems to be particularly effective at reducing cough and the urge-to-cough. Although it seems likely that cortical modulation of the sensory pathways involved in discerning an urge-to-cough are involved in this process, these cortical networks have only just begun to be described in humans and the effect of placebo on urge-to-cough has not yet been studied experimentally.

If urge-to-cough can be reduced by placebo under experimental conditions, this would imply that reports from clinical trials of large placebo effects in antitussive trials are due to a true placebo effect rather than other factors. Additionally, uncovering the functional neuroanatomy underlying placebo downregulation of urge-to-cough may help us to understand the processes that generate abnormal urge-to-cough.

Aims

This project aims to investigate cortical modulation of the perception of airway irritation using fMRI in healthy volunteers. By manipulating the experience of urge-to-cough using a placebo design combined with capsaicin cough challenges; we first attempted to establish that reports of high levels of placebo responses in cough could be replicated experimentally. Secondly, we aimed to use this manipulation to identify cortical and subcortical regions that are involved in regulating the urge-to-cough.

Experiment 1

The first experiment consists of a psychophysical study with the aim of establishing whether it is possible to elicit a robust placebo response in healthy volunteers using a capsaicin model of cough. Our hypothesis is that sensitivity to tussive stimuli, as measured by mean urge-to-cough ratings, will be significantly lower when participants receive a placebo treatment prior to capsaicin inhalation, compared to when they receive no treatment. If this hypothesis is true, then the placebo effect seen in clinical trials might be caused by a decrease in urge-to-cough.

Experiment 2

The second experiment will replicate the previous study during whole brain fMRI, allowing identification of cortical regions of the brain that are involved in regulating aspects of the urge-to-cough. We hypothesise that there will be a decrease in activation in somatosensory areas relating to incoming sensory stimuli from the airways following placebo treatment, indicating that placebo

indeed acts via decreasing the neural response to stimuli. We further hypothesise that other cortical areas, such as the dorsolateral prefrontal cortex, will show an increase in activation during the placebo treatment, indicating a role in inhibitory regulation of the cough pathway. We intend to compare these areas to those known to play a role in placebo analgesia. This will allow us to establish whether there is a common network of regions related to the placebo response in different modalities, or whether there are specific areas related to the control of cough.

Experiment 3

To further investigate this effect, during the third experiment we will use high resolution fMRI optimised for the brainstem to examine processing of tussive stimuli in the brainstem during placebo treatment. We expect to see decreased activation in brainstem areas related to respiratory control, such as the nucleus of the solitary tract. This will allow us to establish whether regulatory cortical areas identified in the previous experiment exert their effect via descending inhibitory modulation of brainstem regions, or whether the inhibition occurs at a purely cortical level.

Significance

The proposed research project is expected to contribute to the understanding of cortical regulation of the perception of airway irritation. While the basic physiology of the cough reflex has been extensively studied using animal models, there is still a lack of knowledge regarding the functional organisation of cortical and brainstem regions involved in modulation of the urge-to-cough network. Given the lack of effective treatments for patients presenting with chronic cough symptoms and evidence suggesting that dysfunction of this regulatory network may be involved, it is hoped that this research could lead to novel therapeutic targets for cough disorders.

Chapter 2 - METHODS - Experimental Design, Functional Magnetic Resonance Imaging and Analysis

Overview of the principles of fMRI

The advent of neuroimaging has greatly enhanced our understanding of the functional neuroanatomy of the brain. Using fMRI it is now possible to visualise areas of the brain that are active whilst a task is being performed. New methods continue to be developed which may lead to further advances in functional brain mapping and clinical applications.

fMRI measures changes in signal intensity over time due to local fluctuations in blood flow. These fluctuations reflect underlying neuronal activity relating to the task being performed (Logothetis, 2002). fMRI offers significant advances in spatial resolution compared to other neuroimaging techniques such as PET and magnetoencephalography (MEG) and has the benefit of not exposing subjects to ionising radiation, allowing for repeat scanning of healthy participants.

MRI was originally used for anatomical investigations, relying on the differing magnetic properties of tissues to produce contrasts between structures. In 1990 a study first described the possibility of using blood oxygenation level as an endogenous contrast agent, based on the differing magnetic properties of haemoglobin (Hgb) and deoxygenated haemoglobin (deHgb) (Ogawa, Lee, Kay, & Tank, 1990). This led to the development of blood oxygenation level dependant (BOLD) MRI, the basis of functional imaging.

Local neuronal activity related to task performance is accompanied by an increase in blood flow, presumably to supply the metabolic needs of neurons and astrocytes at active synapses (Logothetis, 2003; Logothetis & Pfeuffer, 2004). However the increase in oxygen supply exceeds the increase in oxygen consumption (Fox & Raichle, 1986), leading to a greater proportion of Hgb to deHgb in active areas. deHgb interferes with the magnetic field of nearby water molecules, leading to a decrease in the MRI signal detected. Because Hgb does not have the same properties, an increase in the ratio of oxygenated blood leads to an increase in signal shortly after an area has been stimulated. This is known as the haemodynamic response function (HRF). The HRF is coupled to neuronal activity (Raichle & Mintun, 2006) and consists of an initial dip in the BOLD signal, followed by a peak positive signal increase 5-8 seconds following the stimulus. This delay is incorporated into models used in processing data from fMRI studies.

Airway irritations: Modelling cough versus the urge-to-cough

Cough and the urge-to-cough can be induced experimentally in healthy subjects using chemical or mechanical stimuli. Inhaled tussive challenges are the most common method, using stimuli such as capsaicin, citric acid, hypertonic saline or fog (Dicpinigaitis, 2003, 2007). The cough induced by these agents is safe, transient, reproducible and dose-dependant (Dicpinigaitis & Alva, 2005). The C2 threshold (dose of tussive stimuli causing 2 or more coughs) is commonly used as a measure of cough sensitivity. C2 thresholds have now been reported in a number of studies. Although there is a wide range of natural variation between individuals, C2 thresholds are on average lower in women (Dicpinigaitis & Rauf, 1998; Fujimura et al., 1996) and differences in C2 thresholds have been reported in various groups, such as smokers (Kanezaki et al., 2010; Millqvist & Bende, 2001; Sitkauskiene & Dicpinigaitis, 2010) and asthmatics (Jesenak et al., 2009). Although the ability of C2 thresholds alone to discriminate between healthy controls and chronic cough patients on an individual basis is poor (Nieto et al., 2003), there is evidence that successful treatment of chronic cough is associated with an increase in a patient's C2 threshold (Oconnell, Thomas, Pride, & Fuller, 1994).

During inhaled tussive challenges, subjects generally report an urge-to-cough at lower concentrations of stimuli than that which cause coughing (Davenport, 2008; Davenport, Sapienza, & Bolser, 2002). The lowest dose of tussive stimuli to generate a perception of urge-to-cough (Cu) has been proposed as an additional measure of urge-to-cough sensitivity (Davenport et al., 2002; Dicpinigaitis, Rhoton, Bhat, & Negassa, 2012). Like C2, this is a reproducible measure (Dicpinigaitis et al., 2012) and there is a linear relationship between increasing log-transformed concentrations of tussive stimuli and the urge-to-cough (Davenport et al., 2002). Unlike C2, gender differences have not been noted (Dicpinigaitis et al., 2012; Gui et al., 2010), however Cu is transiently enhanced during upper respiratory tract infections in healthy non-smoking volunteers (Dicpinigaitis et al., 2011).

Taken together, this evidence suggests that Cu and C2 are not equivocal, instead there appears to be a complex interaction between these two measures. Cu may in fact be a more appropriate measure of sensory dysfunction such as that seen in chronic cough, as C2 measures not only the urge-to-cough, but also the ability to suppress cough. Taking these two measures together may provide a more nuanced measure of abnormal cough, as together they encompass both the sensory aspect and the motor reflex of cough.

Choosing a Placebo “Treatment”

This paradigm presents certain challenges, as there is only one route of administration for tussive stimuli, in contrast to placebo analgesia studies where different sites on the body (for instance the right hand and left hand) are typically used to compare placebo to no treatment. In analogous fMRI placebo analgesia studies with only one administration route, such as those involving visceral sensations like oesophageal or rectal pain, researchers have typically separated the placebo and control treatments in time, with a no treatment session followed by a placebo analgesia session (Lee et al., 2012; Lu et al., 2010; Price, Craggs, Verne, Perlstein, & Robinson, 2007). This strategy however has the drawback of greatly reducing statistical power.

In developing the protocol for these studies, my aim was to design a study where periods of no treatment and placebo could be interspersed in the same run, allowing for a more statistically powerful block-design fMRI study. For this reason I chose to describe the placebo as an aerosolised local anaesthetic, rather than a more common form of antitussive such as a cough syrup. The only previous study looking at placebo effects in cough informed participants they were testing the effect of vitamin E tablets on cough (Lee et al., 2005). Although this proved successful in evoking a placebo effect, I wished to use a strategy where the treatment would be viewed as plausible, short acting and highly effective at reducing cough, allowing for rapid switching between control and treatment conditions in the same fMRI run.

Aerosolised lidocaine was thus chosen as the placebo “treatment” since both local anaesthetics and aerosolised medications are generally perceived as being effective and short lasting, with a rapid onset and offset. Lidocaine was also chosen as a cough treatment that participants were unlikely to have experienced, removing any negative expectancy due to previous exposure to over-the-counter antitussive medications that may not have been particularly effective. This description served the additional function of being a plausible antitussive, lidocaine being used clinically to inhibit cough, particularly during medical procedures (Trochtenberg, 1994; Truesdale & Jurdi, 2013; Udezue, 2001), as well as being effective at reducing capsaicin-induced cough (Midgren, Hansson, Karlsson, Simonsson, & Persson, 1992). This was an important consideration since participants in the studies were drawn primarily from advertising within the University of Melbourne, and were likely to have access to medical databases should they choose to research the treatment after reading the participant information and consent form and prior to the study. Indeed, a number of the participants recruited were medical or dental students or graduates. This strategy was successful in

that all participants reported during debriefing that they believed they had received an active treatment during the study.

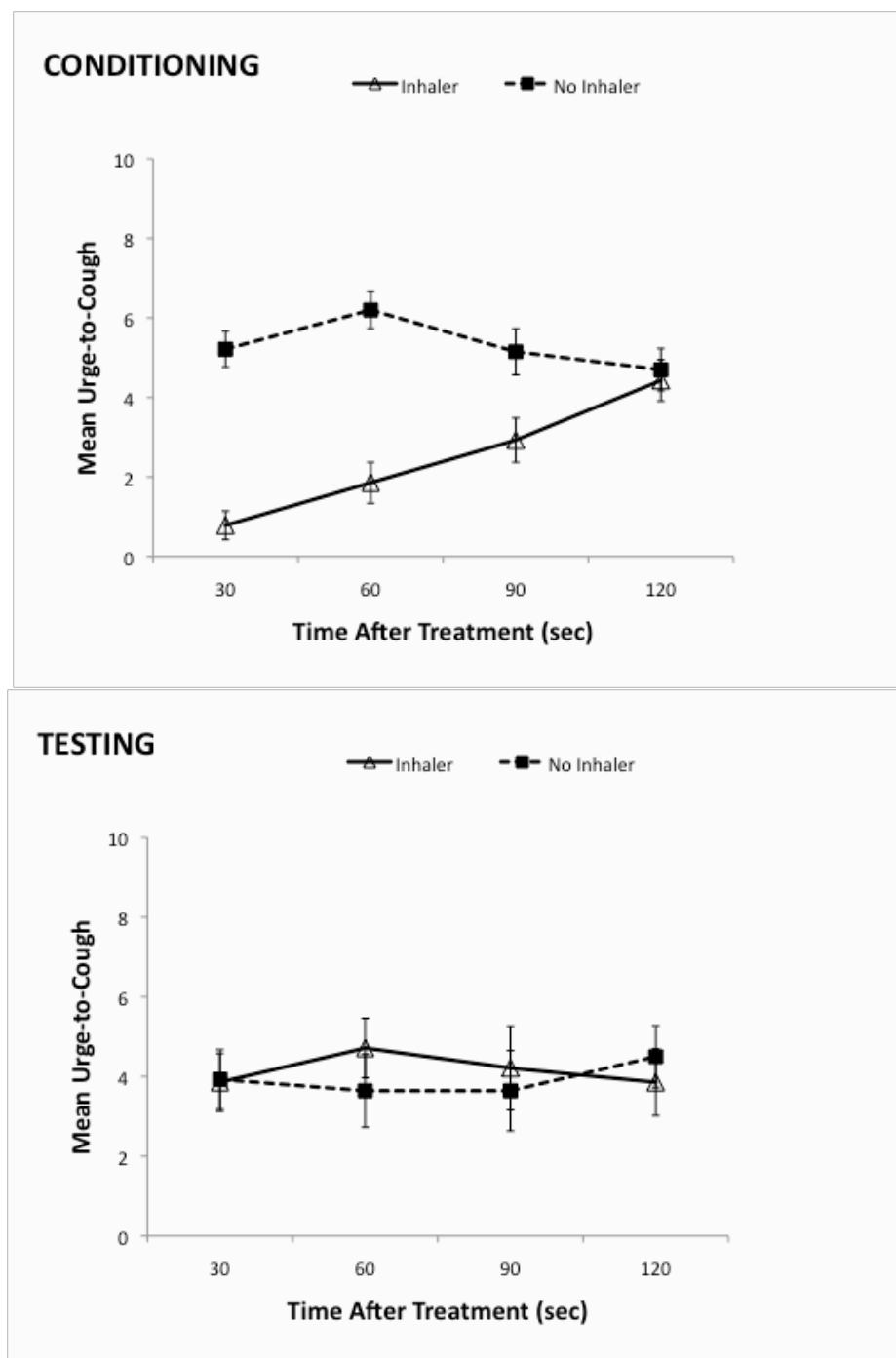
Development of Study Designs

Our group has previously developed a reliable method of delivering aerosolised capsaicin challenges during fMRI scanning (Mazzone et al., 2011; Mazzone et al., 2007). During the scanning session, participants are fitted with a facemask attached to nebulisers containing capsaicin solution. At specific times, capsaicin is delivered by turning on pressurised medical air to the nebulisers, which participants then passively inhale via the facemask. In this series of experiments, we chose to deliver the placebo treatment in a distinctive fashion, rather than simply delivering nebulised saline via the same facemask as the capsaicin, in order to accentuate the difference between placebo treatment and capsaicin inhalation.

In the psychophysical study this was achieved by using a placebo inhaler, a replica metered dose asthma inhaler that delivers a single puff of pressurised gas (Implox Healthcare, Adelaide, Australia). Although the inert propellant gases were not expected to have any effect on urge-to-cough, in order to confirm the fact we explicitly tested this in a separate cohort of subjects who completed the same study without any expectancy, i.e. with the knowledge that the inhaler did not contain any active medication. Results from this study are reported in Chapter 3 and confirmed our expectation that the placebo inhaler alone had no effect on capsaicin induced urge-to-cough.

An initial pilot experiment was conducted in order to establish the feasibility of producing a placebo response using a capsaicin inhalation model. This study was discontinued after seven participants had completed the protocol, due to a lack of observable placebo effect (Figure 4). This group of subjects underwent conditioning, which appeared to be successful, however no steps were taken to enhance expectation, and a number of participants reported during debriefing being suspicious as to whether they were actually receiving an active treatment.

Figure 4 - Effect of Placebo Treatment on Mean Capsaicin-Induced Urge-to-Cough Ratings during Pilot Study



Upper panel: shows results from the conditioning phase in which the challenge concentration of capsaicin was deceptively altered without the participants' knowledge to give the impression that the inhaler treatment produced a transient reduction in urge-to-cough sensation that resolved over time.

Lower panel: shows results from the testing experimental phase in which the challenge concentration of capsaicin was maintained constant for all challenges. Urge-to-cough was measured on a modified Borg scale for subjective ratings 0-10

and inhaler treatment was administered at time = 0. No significant difference in ratings between placebo and no treatment was observed at any of the time points (pairwise t-test, $p=n.s.$). Data are the mean \pm SE of $n=7$ participants.

Following this pilot study, we recruited a new cohort and incorporated strategies into the paradigm to increase participants' expectancy and reduce the chance of them developing suspicions regarding the treatment. These included: showing participants an information leaflet outlining contraindications and possible side effects of lidocaine (see Appendix A); adding professional-looking labelling to the placebo inhaler, including "lidocaine hydrochloride 4%" and "for practitioner use only"; explicitly informing participants that lidocaine is highly effective at reducing the urge to cough; and additionally informing them that the aim of the experiment was not to test the efficacy of lidocaine, but rather to pilot a prospective protocol for later use in a study on chronic cough patients.

In addition, we introduced a series of questionnaires (see Appendix B) to identify whether participants believed the treatment was working (following conditioning) and whether they had any suspicions that they were not receiving an active treatment (following debriefing). After introducing these extra measures, a significant decrease in urge-to-cough ratings was observed in subsequent cohorts. Similarly, a recent study also showed increased placebo analgesia in participants who had received an educational handout about the treatment (Tang & Colagiuri, 2013). This observation underlines the importance of both conditioning and expectation in the elicitation of experimental placebo effects.

Development of Placebo MRI protocol

Whilst effective, this method of lidocaine administration needed to be modified for the following study to meet the restraints of an MRI environment. Namely, the ferromagnetic properties of the pressurised gas canister prohibited its use during scanning. For this reason a nasal cannula was used as the method of placebo delivery during the MRI studies. This allowed us to again differentiate the treatment from the capsaicin inhalation, while still using aerosolised lidocaine as the supposed treatment.

This experiment required some modification of the capsaicin inhalation protocol that our group has previously used during MRI studies (Farrell et al., 2012; Mazzone et al., 2011; Mazzone et al., 2007). In these studies the subject was cued 5 seconds prior to the onset of capsaicin and asked to time their next inhalation to coincide with the onset of capsaicin delivery. This introduced a random period of breath holding into most trials. Even a brief breath hold of this nature has a tendency to create negative signal change in the fMRI data that can persist for up to 20 seconds (Abbott, Opdam, Briellmann, & Jackson, 2005). Normally this would not cause significant issues as the

artefacts introduced are both normally distributed and present in all conditions, thereby not affecting the contrasts of interest e.g. between high dose and low dose capsaicin. It may however lead to less statistical power owing to variation in breath hold depth and length between trials.

In the proposed experiment however, the nasal inhalation would have been required to precede the capsaicin inhalation by more than twenty seconds, or the beginning of the capsaicin inhalation period would be affected by this negative signal change. Introducing a twenty second baseline period would have reduced the number of trials able to be presented within a reasonable amount of time, also lowering statistical power. For this reason it was decided to trial a method where participants were not cued and instead were encouraged to breath normally throughout the experiment while their respiratory cycle was measured by way of a respiratory belt, time locked to both stimulus presentation and scanner acquisition. This allowed for the respiratory phase to be reconstructed relative to the onset and offset of stimuli, and the trial timings to be adjusted accordingly, without the need for breath holds. For instance, if the subject was exhaling as the capsaicin was turned on, timings for statistical analysis were adjusted to begin at the next inhalation. This was achieved using LabChart (ADInstruments; <http://www.adinstruments.com/>) and custom Python scripts (<http://www.python.org/>).

Respiratory related global signal decreases were still observed in the data using this method, likely due to the change in respiratory pattern from normal breathing to nasal breathing, however they were of a lesser magnitude than that observed previously using cued inhalation and, most importantly, they did not differ between control and placebo conditions. As the task is intrinsically linked to respiration, these artefacts are difficult to avoid and additional analysis steps were incorporated in order to minimise their effect.

To account for the residual respiratory noise, additional regressors were incorporated into the general linear model as advocated by Birn et al and successfully implemented in previous studies (Birn, Murphy, Handwerker, & Bandettini, 2009; Farrell et al., 2012; Mazzone et al., 2011). In the whole brain MRI study these included regressors from cerebrospinal fluid (CSF), white matter and voxels with high standard deviation in the sagittal sinus. Signals from these regions reflect physiological noise associated with cardiac and respiratory cycles and would not be expected to show task related changes. This has proven to be equally or more effective than methods such as utilising physiological measurements, such as RETROICOR (Glover, Li, & Ress, 2000). As the haemodynamic response function peaks at roughly 6 seconds post stimulus and the effect of

respiration on the signal peaks up to 20 seconds post stimulus, the loss of power from regressing out possibly task correlated respiratory changes should be minimal.

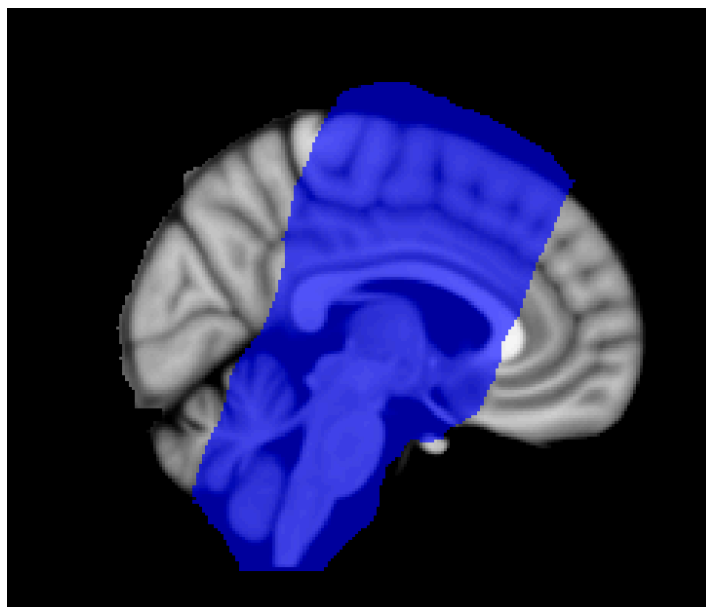
In the whole brain MRI study (Chapter 4) an additional regressor was created to account for these global effects (Andersson, 1997). As the brainstem is less susceptible to low frequency global artefacts, this step was not included in the brainstem analysis (Chapter 5). For the brainstem analysis, regressors were created to minimise high frequency fluctuations from cardiac and respiratory processes, these included time courses from voxels in CSF, voxels of highest standard deviation in the left and right carotid arteries, and voxels with high standard deviation in the sagittal sinus.

Development of Brainstem Placebo MRI protocol

The brainstem fMRI protocol was similar to the whole brain fMRI protocol with the following exceptions: participants completed three runs rather than four in order to minimise total scanning time; similarly, the time between the placebo presentation and capsaicin challenge was reduced, allowing for a greater number of challenges to be presented in the same amount of scanning time. The field of view (FOV) was reduced to 27 slices covering the brainstem, allowing for greater spatial and temporal resolution (See Figure 5).

In the brainstem fMRI experiment, the addition of a line fixed to the nebulisers and connected to a syringe allowed for the adjustment of capsaicin doses from outside of the MRI scanner. This allowed doses to be adjusted as necessary (for instance if the participant became habituated to the stimuli or began to cough during the scan), without the need to withdraw the participant from the MRI scanner. This saved time during the session, as well as removing the need for extra localisation scans, and minimising registration issues to changes in the participant's head position. This relatively simple procedure would be recommended for any future MRI studies utilising nebulised capsaicin.

Figure 5 - Field of View for Brainstem fMRI Study



Sagittal cross section showing average field of view for all participants (in blue) optimised for brainstem imaging, overlaid on a standard MNI template.

Subcortical MRI imaging presents particular challenges due to both the scarcity of human research accurately localising brainstem nuclei and the higher signal variability in brainstem regions. Signal to noise ratios in the brainstem are lower than in the cortex due to the proximity of tissue boundaries which differ in their magnetic susceptibility, introducing distortions into the magnetic field. Temporal variation in physiological processes also increases signal variability. Cerebrospinal fluid flow, respiratory cycle and cardiac pulsations due to the proximity of both the trachea and large veins to the region of interest all contribute to image distortion and increased levels of noise in fMRI studies of brainstem processing (Barry et al., 2013).

Additionally, registration of individual brains to a standard template, as implemented in all currently available fMRI analysis software, is optimised for the cortex rather than subcortical regions. For this reason we used a multi-stage registration process, incorporating as a first step registration to a whole brain EPI image at the same resolution as the functional images, before registration to an individual high resolution T1 image, which was in turn registered to a standard MNI template. This approach has been shown to improve image registration when working with a FOV containing limited numbers of slices (Limbrick-Oldfield et al., 2012).

Although probabilistic atlases incorporating the cerebellum have recently become available (Diedrichsen, Balsters, Flavell, Cussans, & Ramnani, 2009; Diedrichsen et al., 2011), standardised

MRI templates incorporating atlas tools are yet to be developed for regions in the medulla and pons. Given the recent advances in high-resolution 7T fMRI scanning and a concurrent increase in the number of studies looking at small brainstem or spinal cord nuclei, research into tools for automated registration and localisation of subcortical regions would be of great benefit for future research.

As information regarding the potential regions involved in capsaicin-related urge-to-cough suppression is limited, we chose to use a relatively large region of interest based on results from the whole brain MRI study, rather than the alternative option of restricting analyses to a priori regions based on anatomy. While this may somewhat reduce statistical power, as potential regions of interest cannot be defined based on individual anatomy, it has the advantage of being inclusive (Napadow, Dhond, Kennedy, Hui, & Makris, 2006). This allows for identification of activation in unexpected regions, as well as being more robust with respect to individual variation in brainstem structure and accuracy of registration to a standard template.

The region of interest was thus taken from a summary map of brainstem areas activated during any condition (control, placebo or conditioning) in the whole brain study. This was used in conjunction with FSL Randomise to produce activation maps restricted to relevant regions in the brainstem. This not only confined analysis to the relevant regions, but also restricted analysis to regions expected to show increases in activation relative to baseline, reducing the chance of spurious “activations” due to relative changes in deactivation.

Summary

Capsaicin inhalation challenge is a reliable method of inducing urge-to-cough in healthy volunteers. Expanding on our previously developed method of delivering aerosolised capsaicin during fMRI scanning, the following studies employ a novel method of non-cued inhalation periods. Placebo suppression of the capsaicin induced urge-to-cough was achieved by a combination of expectation and conditioning. Describing the placebo treatment as an aerosolised local anaesthetic allowed for alternation between placebo and no treatment trials in the same block design fMRI run, increasing the statistical power of the design.

Chapter 3 - The Effect of Placebo Conditioning on Capsaicin-Evoked Urge-to-Cough

Abstract

Background: The urge to cough is a clinical symptom of respiratory disease that precedes the motor act of coughing. Although previous studies have shown that cough is particularly susceptible to placebo suppression, it is unclear whether the perception of an urge to cough is also modifiable by placebo. Therefore, we tested the hypothesis that capsaicin-evoked urge to cough could be suppressed by placebo conditioning.

Methods: Eleven healthy participants were unknowingly conditioned to believe that an inert inhaler temporarily suppressed capsaicin-induced urge to cough by deceptively modifying the challenge concentration of capsaicin. In subsequent testing, capsaicin-evoked urge-to-cough subjective ratings were assessed in four challenges with a single dose of inhaled capsaicin following no treatment or the placebo metered-dose inhaler. An additional 10 participants were informed that the inhaler therapy was inert prior to receiving capsaicin challenges with and without inhaler treatment.

Results: There was a significant decrease in mean urge-to-cough ratings to capsaicin challenge following placebo compared with no treatment followed by capsaicin challenge ($P < .001$), with a peak decrease of 45%. The placebo inhaler alone had no effect on urge-to-cough subjective ratings when participants were aware that it contained no active medication.

Conclusions: These data confirm that the urge to cough is susceptible to placebo inhibition. This provides further evidence that higher brain networks are involved in the processing of respiratory sensations related to airway irritation.

Introduction

Although coughing can be initiated via a brainstem reflex independent of higher order neural control, this may not be the case in cough disorders in which excessive coughing, behavioural coughing and the persistent desire to cough (urge-to-cough) are hallmark characteristics (Davenport, 2009; McGarvey & Morice, 2006). Studies from our group have described higher brain networks involved in the sensory and motor control of cough in humans (Mazzone et al., 2011; Mazzone, McGovern, Koo, & Farrell, 2009; Mazzone et al., 2007). These studies highlight the complexity of cough neural circuitry and argue against the concept that coughing is purely a reflexive response to airway irritation. Consistent with this, cough is highly susceptible to placebo suppression. Indeed, many currently available antitussive therapies, including codeine and dextromethorphan, offer minimal therapeutic effect over placebo in treating cough (Eccles et al., 1992; Lee et al., 2005; Smith, Owen, Earis, & Woodcock, 2006; Tukiainen et al., 1986). Given that antitussive therapies provide symptom relief, these observations suggest a high propensity of cough neural pathways to be regulated by higher order inhibitory control mechanisms.

The mechanisms by which placebo suppresses coughing are poorly described. It has been speculated that antitussive medications may work either because of a true placebo effect (Eccles, 2002) or as a consequence of non-pharmacologic agents (such as sugar) in the antitussive formulations, which may act to recruit endorphin or opioidergic circuitry in the brain (Fuller & Jackson, 1990). Regardless of the mechanism of action, placebo groups in antitussive clinical trials often show improvements in outcome measures above what would be expected as a result of spontaneous resolution of symptoms (Birring, 2009). For example, a meta analysis of antitussive clinical trials for upper respiratory tract infection reported a mean placebo response rate of 85% relative to the active medication groups (Eccles, 2002). The importance of placebo effects in antitussive clinical trials and the need for further research in this area has been widely noted (Birring, 2009; Eccles, 2002, 2010). Large placebo effects may obscure the clinical benefit of treatments under investigation (Temple & Ellenberg, 2000; Temple, 1997), resulting in therapies that appeared promising in animal experiments failing to reduce cough over placebo in clinical trials, even though they may be more effective than no treatment in clinical practice (Meissner, Kohls, & Colloca, 2011b).

Although available evidence suggests that both placebo intervention and voluntary control mechanisms can suppress the motor act of coughing (Hutchings, Morris, Eccles, & Jawad, 1993; Lee et al., 2005), it is unclear if the sensory perception of airway irritation is similarly susceptible to higher order inhibitory control. Indeed, no studies have explicitly investigated the effects of placebo

on the urge-to-cough, a measure of airway sensation.

The aim of the current study was therefore to determine whether placebo intervention would decrease the subjective sensation of airway irritation in healthy humans.

Methods

Participants

This study was approved by the Melbourne Health Human Research Ethics Committee (approval number 2010-073). A total of 21 healthy adult volunteers were recruited. Participants were excluded from the study if they had a history of chronic respiratory disease or gastroesophageal reflux, or a recent history of acute respiratory infection (minimum of 6 weeks symptom free) or had smoked in the past 6 months. Studies of placebo responses often necessitate participant deception. Information and consent procedures were therefore developed in consultation with our institutional ethics committee with the objective of sustaining a viable experimental model while acknowledging concerns about informed consent. All participants gave written informed consent using a modified consent form, in which the general nature of all procedures and risks involved in participating in the study were detailed, but the aim of testing a placebo was omitted. A written and verbal debriefing was provided to participants at the conclusion of the experimental session to disclose the deceptive nature of the study and to explain in detail the true aims and methods used and the reasons for including deception in the study design. A second informed consent was then sought from participants to use their data collected during the experiment. No participant chose to exclude their data from the protocol.

Capsaicin sensitivity testing

This study assessed subjective reports of the urge-to-cough evoked by inhalation of capsaicin, a chemical that causes transient airway irritation and is widely used to investigate cough (Dicpinigaitis, 2007; Dicpinigaitis & Alva, 2005). Capsaicin challenges were delivered via pressure driven nebulisers (RapidFlo, Alkaline Corporation) connected to an air compressor (flow rate 0.42 ml/min; Liberty Healthcare Corporation). Capsaicin concentrations were prepared in doubling doses from 0.25 μ M (Mazzone et al., 2007), and participants were challenged with a random series of single vital capacity inhalations to determine their individual sensitivity. After each inhalation participants were asked to rate their urge-to-cough on a modified Borg scale ranging from 0 (no discernible urge) to 10 (maximal urge). The capsaicin concentrations that resulted in the first

perceivable urge-to-cough and that first evoked two or more coughs were defined as the urge-to-cough (Cu) and cough (C2) threshold concentrations, respectively (Davenport, 2009; Diczpinigaitis et al., 2012). A moderate stimulus intensity (MI) was defined as a dose of capsaicin causing an average urge-to-cough rating of 6.

Expectation

Participants (n = 11) were informed via a scripted dialogue that the aim of the study was to assess the length of time that a treatment would reduce their urge-to-cough in response to capsaicin challenge. Participants were told they would receive lidocaine hydrochloride, a commonly used local anaesthetic that would be highly effective at reducing the urge-to-cough for a short period of time. The placebo “lidocaine” was delivered via self-administration of one puff of a placebo metered dose inhaler (Implox Pty Ltd), professionally labelled as “4% lidocaine hydrochloride” but containing only inert propellant gases. Participants were also shown an information leaflet outlining possible side effects and contraindications to reinforce the deception. An inhaler was chosen as the placebo intervention because it was unlikely to have been encountered by healthy participants in this context, avoiding the possibility of negative expectations due to previous use of non-prescription antitussives which could have reduced the effect of conditioning (Colloca & Benedetti, 2006). Additionally, inhaled medications and local anaesthetics are often associated with a short duration of effect, allowing for a within subject cross-over design.

Conditioning

Participants were led to expect that the treatment would be effective but would wear off over a period of approximately 2 minutes. This deception was reinforced by informing participants that they would receive either “lidocaine”, or no treatment, followed by a series of four vital capacity breath MI concentration capsaicin challenges, 30 seconds apart. During no treatment trials participants indeed received four challenges with their individualised MI capsaicin concentration. However during placebo trials the capsaicin concentrations were lowered without participants’ knowledge to one eighth, one quarter and one half the MI dose for the first three challenges respectively. The fourth challenge was at the MI dose to reinforce the transient nature of the treatment. During conditioning, two no treatment and two placebo trials were conducted in a randomised order, with 1 minute intervals between trials.

Testing

During the testing phase, a further two placebo and two no treatment trials were conducted as

described previously, with the exception that the MI concentration was administered during all capsaicin challenges. Subjects were not aware that these trials varied from the preceding ones. Following the conclusion of testing, participants were asked whether they thought the treatment was effective. After debriefing, participants were asked if they had had any suspicions before or during the study that the treatment was a placebo.

Effect of metered dose inhaler on urge-to-cough thresholds

To confirm that the inert propellant gases in the metered dose inhaler did not affect capsaicin evoked urge-to-cough, a separate experiment was conducted in a second cohort of participants (n=10) who were aware that the inhaler contained inert gases but were unaware of the expected lack of effect of these gases on capsaicin urge-to-cough ratings. Thirty seconds after receiving either no treatment or the metered dose inhaler, participants were administered a single breath capsaicin challenge of one of three different doses. The doses presented were low (one half the MI capsaicin concentration), moderate (MI capsaicin concentration), or high (double the MI capsaicin concentration), based on each individual's capsaicin sensitivity. Twelve randomised trials were presented, consisting of four instances of each dose, preceded by the metered dose inhaler on one half the occasions. Participants were asked to rate their urge-to-cough following each capsaicin challenge as described previously.

Statistical Methods

All analyses were carried out with PASW statistics (Release Version 18.0.0, SPSS, Inc., 2009, Chicago, IL, www.spss.com). Attributes of the sample were characterised with descriptive statistics. The doses required to elicit Cu, C2 and MI were tabulated for each participant and the geometric means were calculated by logarithmic conversions of the doses averaged across the sample. Multivariate comparisons were performed using repeated measures analysis of variance (ANOVA). Urge-to-cough ratings were averaged across trials for each challenge and condition for each participant and these mean ratings were used to test for the effects of conditioning (conditioning versus testing), treatment (placebo versus no treatment), challenge (four serial capsaicin challenges) and the interactions of the factors. Another ANOVA was used to assess the effects of inhaler, stimulus dose and their interaction on the urge-to-cough ratings of the second cohort of participants. Post hoc testing of paired observations was performed with dependent t-tests; $p < 0.05$ was considered significant.

Results

Participants

Eleven healthy volunteers aged between 19 and 42 were recruited for testing placebo evoked suppression of the urge-to-cough (age, 27.3 ± 6.9 , 4 males). Ten additional volunteers (age, 31.7 ± 9.2 years, 4 males) were recruited to test the effect of the metered dose inhaler on urge-to-cough ratings.

Thresholds and Urge-to-Cough Ratings

The Cu, C2 and MI thresholds and geometric means are reported for each participant in Table 1. Repeated measures ANOVA of urge-to-cough ratings showed significant within subject effects for the three main factors of conditioning ($F(1,10) = 5.2$, $p < 0.05$), treatment ($F(1,10) = 102.0$, $p < 0.001$) and challenge ($F(3,30) = 15.6$, $p < 0.001$). Two-way interactions between the main factors were also significant (conditioning by treatment [$F(1,10) = 8.1$, $p < 0.05$], conditioning by challenge [$F(3,30) = 4.8$, $p < 0.001$], treatment by challenge [$F(3,30) = 21.6$, $p < 0.001$]), as was the three-way interaction ($F(3,30) = 7.4$, $p < 0.001$). A tendency for urge-to-cough ratings to generally decrease in magnitude across the course of the experimental protocol explained in part the significant effects of conditioning and its interaction with the inhaler. When expressed as proportional effects, placebo was associated with a 37.9% decrease in urge-to-cough ratings during the conditioning phase and a 28.3% decrease in the testing phase.

Differences in the effects of treatment between conditioning and testing during successive challenges accounted for the remaining two and three way interactions. The first three capsaicin challenges during conditioning were associated with significantly decreased ratings of the urge-to-cough after placebo (challenge 1 $t(10) = 9.9$, $p < 0.001$, challenge 2 $t(10) = 6.6$, $p < 0.001$, challenge 3 $t(10) = 2.7$, $p < 0.05$; Figure 6). This is consistent with the conditioning strategy of administering lower capsaicin doses during the first three challenges. Successively increasing doses after placebo during conditioning led to graduated responses as indicated by significant pairwise increases of urge-to-cough ratings across the three initial challenges (challenge 1 versus 2 $t(10) = 6.3$, $p < 0.001$, challenge 2 versus 3 $t(10) = 5.1$, $p < 0.001$, challenge 3 versus 4 $t(10) = 7.9$, $p < 0.001$).

Table 1 - Individual Capsaicin Sensitivity Thresholds

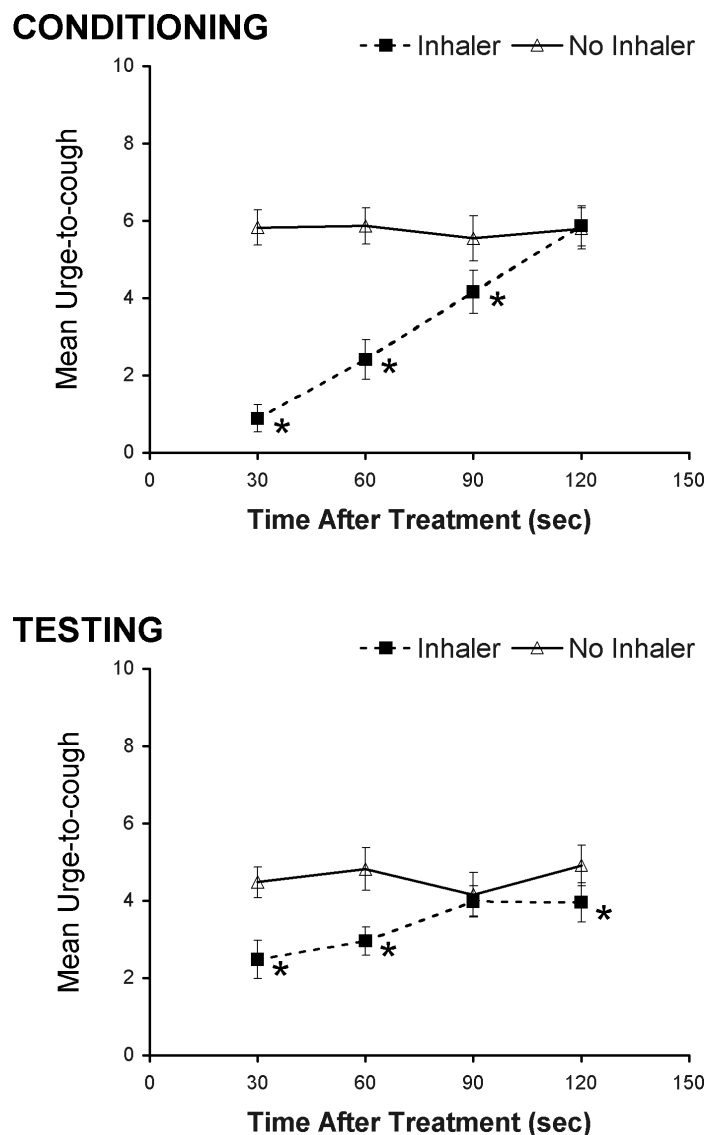
ID No	Cu (μ M)	MI (μ M)	C2 (μ M)
1	0.98	31.25	62.5
2	0.98	7.81	7.81
3	0.98	15.63	31.25
4	1.95	31.25	- ^a
5	0.98	3.91	0.98
6	0.98	7.81	3.91
7	0.98	3.91	0.98
9	0.98	1.95	3.91
9	1.95	31.25	31.25
10	0.98	7.81	3.91
11	0.98	7.81	1.95
Mean \pm S.D.	1.16 \pm 0.39	13.67 \pm 11.82	14.85 \pm 20.46

^a One participant did not have a C2 event at any of the concentrations tested

Abbreviations: Cu: Lowest concentration at which a noticeable perception of urge-to-cough was first recorded. MI: Concentration that was rated closest on average to 6 on the scale of urge-to-cough after multiple single breath challenges. C2: Lowest concentration which first produced 2 or more coughs.

Examination of the urge-to-cough ratings of individual participants during the testing phase indicates that the placebo effect was more consistent during the first two challenges (Figure 7). Only one participant in the first challenge and two participants in the second challenge failed to demonstrate a lower urge-to-cough rating after placebo compared with the no treatment trials. The reductions in urge-to-cough ratings following placebo during the testing phase for the first two challenges were 44.7% and 38.7%, respectively. During the testing phase all 11 participants had a lower mean score for the combined challenges after placebo compared to no treatment.

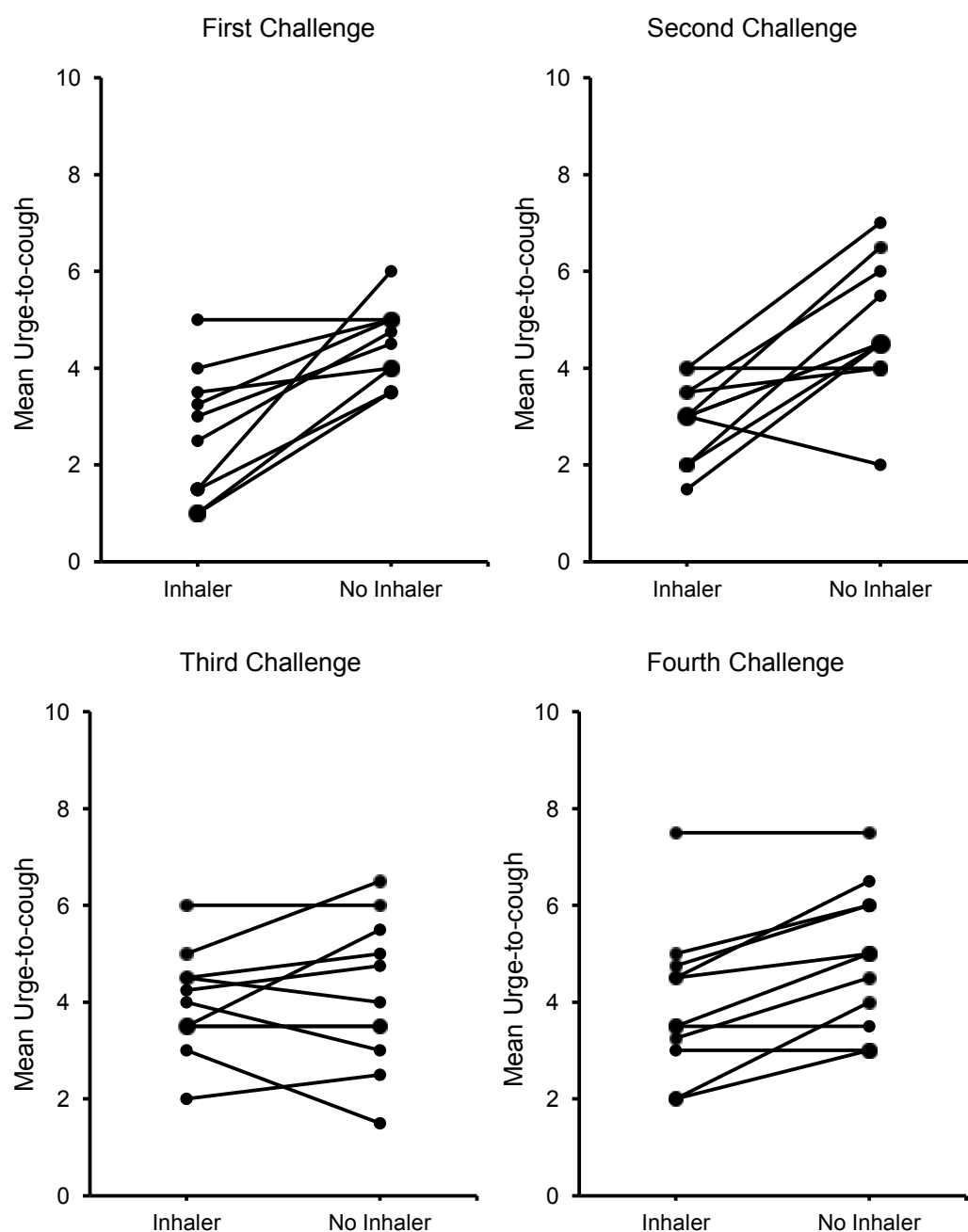
Figure 6 - Effect of Placebo Treatment on Mean Capsaicin-Induced Urge-to-Cough Rating



Upper panel: shows results from the conditioning experimental phase in which the challenge concentration of capsaicin was deceptively altered without the participants' knowledge to give the impression that the inhaler treatment produced a transient reduction in urge-to-cough sensation that resolved over time. Lower panel: shows results from the testing experimental phase in which the challenge concentration of capsaicin was maintained constant for all challenges. In this panel, the successful demonstration of a placebo effect can be seen at 30, 60

and 120 seconds after inhaler treatment. Urge-to-cough measured on a modified Borg scale for subjective ratings 0-10 and inhaler treatment was administered at time = 0 (see text for details). An overall decrease in ratings between the conditioning and testing phases was observed. Asterisks represent significant differences between placebo and no treatment (pairwise t-test, $p < 0.05$). Data are the mean \pm SE of $n=11$ participants.

Figure 7 - Effect of Placebo Treatment on Individual Urge-to-Cough Ratings

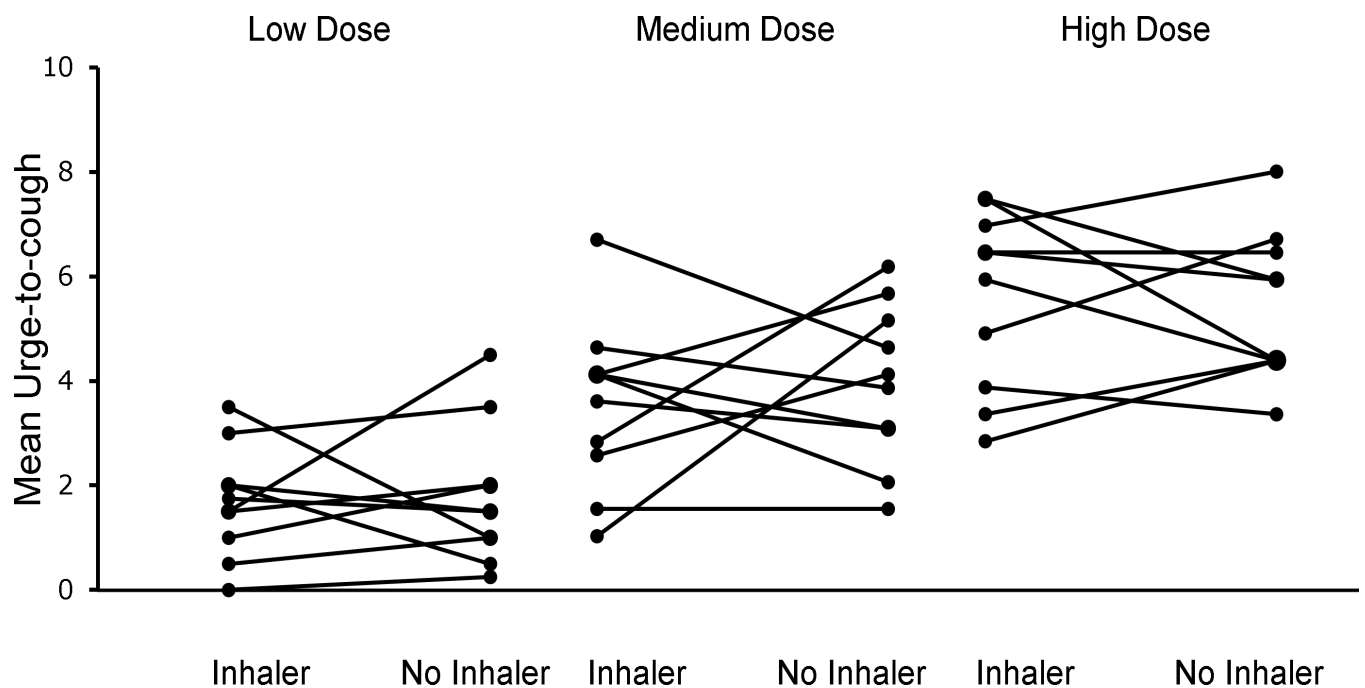


Mean urge-to-cough ratings are shown for each individual during the testing experimental phase. Participants ($n=11$) received the same capsaicin doses for each challenge following either placebo inhaler or no treatment at time = 0. Panels A-D show mean urge-to-cough ratings from each participant following capsaicin challenge at 30, 60, 90 and 120 seconds, respectively. Larger markers at datapoints indicate multiple subjects with the same mean rating.

Effect of metered dose inhaler on urge-to-cough ratings

There was no significant difference between capsaicin evoked urge-to-cough ratings following inhaler versus no inhaler when subjects were aware that the inhaler did not contain any active medication ($F(1,9) = 0.128$, $p=0.729$). There was a significant effect of capsaicin dose ($F(2,18) = 32.35$, $p<0.001$), but no significant interaction between dose and intervention ($F(2,18)=0.27$, $p=0.766$) (Figure 8).

Figure 8 - Effect of Inhaler without Expectation on Capsaicin-Induced Urge-to-Cough



Mean urge-to-cough ratings for individual participants ($n=10$) when they were aware that the placebo inhaler contained no active medication. Results shown are mean urge-to-cough ratings for low, medium and high dose capsaicin challenge following either inhaler or no treatment. There was no significant difference between urge-to-cough ratings following inhaler or no treatment ($p=0.7$). Larger markers at datapoints indicate multiple subjects with the same mean rating.

Debriefing

No participants reported suspicions that the treatment was inactive or did not contain lidocaine and none were aware prior to debriefing that the study was investigating placebo effects. All participants reported thinking that the treatment was effective at reducing the urge-to-cough.

Discussion

This study has demonstrated that conditioning and an expectation of relief are sufficient to produce

substantial reductions in urge-to-cough ratings. This effect was consistent, with all participants reporting decreased levels of the urge-to-cough in response to capsaicin challenges after inhalation of an inert propellant gas that was erroneously ascribed with antitussive properties. The consistency and magnitude of effects associated with deception in this study show that the urge-to-cough, like the cough motor event, is influenced by placebo. Furthermore, the findings provide additional support for the proposition that cough and attendant sensations are subject to modulation by suprapontine brain mechanisms.

The urge-to-cough is a subjective experience represented in distributed brain regions that respond to stimulation of the airways (Mazzone et al., 2009; Mazzone et al., 2007). Our current understanding of the urge-to-cough is that it subserves a motivation to action function, informing the higher brain about the presence of irritants in the airways (Davenport, 2008). In this sense, the urge-to-cough may be important in the behavioural regulation of cough, which might otherwise proceed in a purely reflexive manner. This study shows that in addition to attentional focus, emotion and learning (Van den Bergh et al., 2012), participant belief in urge-to-cough suppression (i.e., placebo) can modulate the sensory experience. Interestingly, the magnitude of the effect seen in the current study is large compared with results from studies of placebo responses in other systems (Pollo, Carlino, & Benedetti, 2011; Price, Riley, & Vase, 2003; Vase, Petersen, Riley, & Price, 2009), despite the fact that habituation to the stimuli over the course of the experiment, as shown by a general decrease in urge-to-cough ratings over time, may have led to an underestimation of the effect size. This suggests that the urge-to-cough may be particularly susceptible to modulation by subjective belief, which is consistent with evidence from clinical trials reporting large placebo responses in measures of cough symptom improvement (Eccles, 2002).

Although the outcomes of this study suggest that central processing of input from the airways can be influenced by cognitive factors, the mechanisms involved in this modulation are not known. Conditioning and beliefs consistent with relief from the urge-to-cough may lead to recruitment of neural circuits that modify the processing of airway sensations. There is a growing awareness of the pathways that contribute to processing of inputs from chemosensitive and mechanosensitive primary afferents in the airways (McGovern, Davis-Poynter, Farrell, & Mazzone, 2012) and these pathways could provide prospective targets for top-down modulation. Comparable studies investigating brain responses during noxious somatosensory stimuli using functional brain imaging have shown that placebo treatment not only reduces participants' subsequent pain ratings, but also decreases activation in pain-related brain regions including the insula, thalamus, anterior cingulate and somatosensory cortices (Bingel, Lorenz, Schoell, Weiller, & Buchel, 2006; Price et al., 2007;

Wager et al., 2004). Thus, top-down modulation of processing of airway inputs would be analogous to the descending inhibitory circuits that have been implicated in placebo effects on pain reports in humans (Eippert et al., 2009a; Eippert et al., 2009b).

Because the urge-to-cough can be a troubling symptom in its own right, top-down circuits that modulate the processing of airway sensory inputs would be a welcome prospect for the development of future therapies should such putative mechanisms find empiric support. Given the outcomes of this study, the combination of placebo treatment of the urge-to-cough with functional brain imaging in humans could provide a viable experimental approach to identify endogenous mechanisms for the relief of sensations arising from airway irritation.

Previous studies have elegantly shown that the motor act of coughing is susceptible to conscious suppressive control mechanisms (Hutchings et al., 1993). Indeed, we have previously reported the neural networks in humans that we believe to be involved in voluntary motor suppression of cough (Mazzone et al., 2011). It is unclear whether the placebo evoked suppression of urge-to-cough noted in the current study similarly represents voluntary control of stimulus sensory perception or urge-to-cough generation. Although previous studies of cough motor suppression have not rigorously reported urge-to-cough scores, we have recently shown that the urge-to-cough persists during voluntary suppression of cough at threshold doses of capsaicin (Farrell et al., 2012). This would argue against the existence of a common neural network that is responsible for motor cough inhibition and placebo evoked regulation of the urge-to-cough. Comparisons of the network components involved in these two suppressive events will help shed light on this question.

It is also conceivable that non-specific factors could influence subjective reporting without impacting the processing of inputs from the airways. For instance, participants could appraise their sensory experiences depending on context so as to label their urge-to-cough as less intense when such a reduction was consistent with their expectations, or the expectations of the researchers. Anxiety may also shape responses to capsaicin challenge. Others have shown that anxiety can influence the experience of respiratory sensations, including the urge-to-cough (Davenport et al., 2009; von Leupoldt, Chan, Bradley, Lang, & Davenport, 2011). The experimental protocol used in this study could produce systematic differences in anxiety levels if the expectation of relief after placebo reduced anxiety about inhaling capsaicin. Finally, it must be acknowledged that our data are specific to capsaicin-evoked urge-to-cough at moderate stimulus intensities. It is unclear at present whether our findings can be extended to other cough modalities (e.g., mechanical, citric acid, fog, and so forth) or to stimulus intensities beyond the moderate range.

Conclusions

Placebo administration alone produced a peak 45 percent reduction in the urge-to-cough, supporting existing evidence that cortical brain mechanisms regulate both the sensory and the motor control of coughing. Understanding the neurophysiologic basis of the endogenous mechanisms of placebo cough suppression may lead to novel therapeutic targets for the treatment of excessive cough in disease.

Chapter 4 - Brain activity associated with placebo suppression of the urge-to-cough in humans

Abstract

Rationale: Antitussive therapies are accompanied by a substantial placebo effect, indicating that inhibitory circuits in the brain have a significant capacity to regulate cough neural processing. However, essentially nothing is known about the identity of these inhibitory circuits or how they reduce coughing. Understanding these processes may help develop more effective antitussive therapies in the future.

Objectives: To identify regional changes in human brain activity related to the urge-to-cough following placebo antitussive administration.

Methods: Seventeen healthy participants undertook functional magnetic resonance imaging while completing a series of inhalations of capsaicin to induce the urge-to-cough. The resultant brain responses associated with capsaicin inhalation without any treatment were compared to those induced by capsaicin following placebo antitussive administration.

Measurements and Main Results: There was a significant decrease in participants' ratings of urge-to-cough following the placebo antitussive administration. Brain activity associated with capsaicin inhalation was less in the somatosensory, primary motor, insula and cingulate cortices during placebo antitussive trials compared to no treatment controls. By contrast, placebo trials were associated with increased activation in the prefrontal and left parietal cortices.

Conclusions: Placebo-related decreases in urge-to-cough are accompanied by commensurate decreases in several brain regions activated during capsaicin inhalation, suggesting that beliefs about treatment can modify the central processing of inputs arising from the airways. The prefrontal cortex and posterior parietal cortex are likely to play an active role in the modification of airway sensory processing after administration of a placebo.

Introduction

The urge-to-cough is recognised as an important clinical symptom that accompanies chronic cough hypersensitivity disorders (Chung, 2011; Morice, McGarvey, & Dicpinigaitis, 2012). It is characterised by sensory nerve-dependent perception of airways irritation and the resultant desire to cough. Understanding mechanisms that regulate urge-to-cough may therefore reveal opportunities to help relieve chronic cough in respiratory disease.

Like cough, the urge-to-cough is subject to regulatory processes that play an important role in shaping its magnitude or characteristics. For example, coughing and the urge-to-cough are markedly reduced by placebo antitussive treatments, suggesting the presence of inhibitory neural pathways that can modify processing of airway sensory inputs. In both clinical and experimental settings the magnitude of this reduction appears to be greater and more consistent than in placebo analgesia (Hróbjartsson & Gøtzsche, 2006; Vase et al., 2009), although the reasons for this are as yet unclear. Eccles (Eccles, 2002) concluded that the placebo antitussive effect is associated with improvements in cough-related symptoms averaging 85% of the benefits seen for active treatments in clinical trials. Perhaps most tellingly, we have shown that a placebo antitussive reduces the urge-to-cough by 45% in healthy people inhaling a tussive agent under controlled experimental conditions (Leech, Mazzone, & Farrell, 2012). It seems plausible that this reduction in urge-to-cough contributes significantly to the effectiveness of placebos and/or therapeutics in reducing coughing in disease. Therefore, understanding the mechanisms underlying these large placebo antitussive effects could have important implications for future development of active antitussive treatments.

Despite repeated observations of an unusually large placebo antitussive response there has surprisingly been no published research into the underlying neurological basis of this effect. We have previously described the neural networks involved in generating the urge-to-cough in humans using functional brain imaging (Mazzone et al., 2007), showing regional brain responses that are related to the intensity of tussive stimuli and magnitude of the perceived urge-to-cough (Farrell et al., 2012). A logical prediction is that these brain responses should show selective decreases following placebo antitussive administration corresponding with changes in the perceived urge-to-cough. Therefore, in the present study we set out to define the effects of placebo antitussive administration on the cough neural network and describe possible brain regions that generate placebo modulation of the urge-to-cough in humans.

Methods

Participants

Healthy adult volunteers with no history of smoking, chronic respiratory disease or recent respiratory infections were recruited. One subject was excluded due to gross neurological abnormalities identified during MRI, which would have precluded accurate registration and interpretation of imaging data. Data analysis was performed on the remaining 17 subjects (10 male, mean age $23.4 \pm \text{SD } 6.6$ years). Written informed consent was obtained via a modified “authorised deception” consent form (Miller, Wendler, & Swartzman, 2005). A full debriefing was provided to participants at the conclusion of each session and they were given the option to withdraw their data if they wished. No participant chose to use this option. All aspects of this study were approved by the Melbourne Health Human Research Ethics Committee (2011.100).

Capsaicin sensitivity testing

Individual cough thresholds were determined using single maximum capacity inhalations of doubling doses of nebulised capsaicin (Leech et al., 2012; Mazzone et al., 2011). After each inhalation participants rated their urge-to-cough on a modified Borg scale ranging from zero (no discernible urge) to ten (maximal urge). The ‘high dose’ of capsaicin for each individual was defined as the highest concentration that evoked a strong urge-to-cough but was not associated with reflex coughing when repeatedly inhaled during 16 seconds of continuous stimulation. The ‘low dose’ was one quarter of this concentration and used for conditioning as described below.

Expectation, conditioning and testing

As previously described (Leech et al., 2012), participants were informed they would on occasion receive lidocaine hydrochloride, a commonly used local anaesthetic that would substantially but briefly reduce their urge-to-cough during capsaicin challenges. The expectation of receiving an active treatment was enhanced using an information leaflet outlining possible side effects and contraindications of lidocaine. Participants were informed that they would receive capsaicin to induce the urge-to-cough and that there would be two types of trials: “lidocaine” and “no treatment”, differentiated by instructions projected on a screen in either green or red text, respectively. The “lidocaine” was in fact normal air (i.e., placebo antitussive), passively inhaled via a nasal cannula.

Participants were informed that the aim of the study was to test a prospective experimental protocol for use in chronic cough patients. Prior to the MRI session, participants completed 2 conditioning runs, each run comprising 3 control ('high dose' capsaicin and no expectation of treatment) and 3 conditioning trials ('low dose' capsaicin preceded by placebo antitussive). Participants believed that they were receiving the 'high dose' on all trials and were informed that this session was aimed at familiarising them with the protocol.

To control for breathing related artefacts, participants were instructed to use the same pattern of inhalation during no treatment trials. During conditioning placebo trials, participants unknowingly received the 'low dose' of capsaicin. During functional MRI the 'high dose' of capsaicin was given for all trials. Following the session participants were asked if they suspected they were not actually receiving lidocaine. All participants believed they received an active treatment.

Image Acquisition

Images were acquired with a Siemens Trio 3 Tesla scanner at the Murdoch Children's Research Institute (Melbourne, Australia). Anatomical T1-weighted sagittal images were acquired for registration purposes (TR=1900 ms; TE=2.59 ms; 192 slices; 0.9mm thickness, 0.8 by 0.8mm in-plane resolution). Functional images were obtained using an echo-planar imaging sequence (TR=2000 ms, TE=32 ms, slice thickness =4.50, 3.28 by 3.28 mm in-plane resolution, flip angle=90 degrees, 216 volumes).

Experimental Protocol

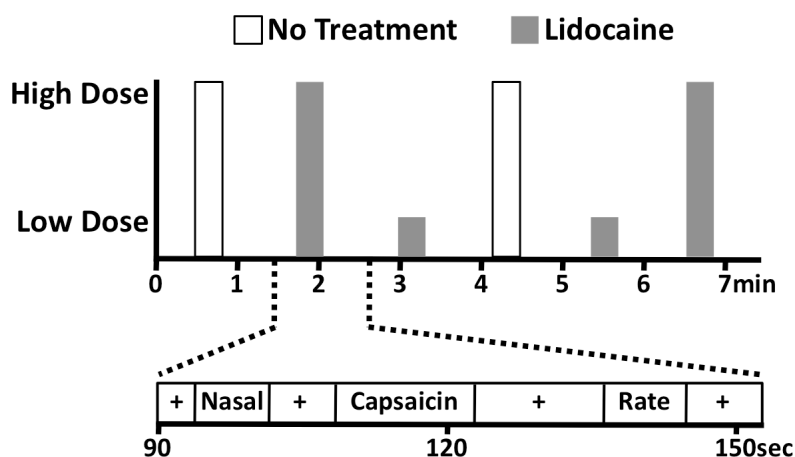
During MRI sessions, participants completed 4 runs of 7min 12sec duration. Runs contained 2 presentations each of control ('high dose' capsaicin and no expectation of treatment), placebo ('high dose' capsaicin preceded by placebo antitussive) and conditioning ('low dose' capsaicin preceded by placebo antitussive) trials, presented in a randomised order. Additional conditioning runs were included during the MRI session in order to sustain the placebo effect over the course of the scanning.

During MRI scanning, participants were given on-screen instructions to rate their urge-to-cough following each trial by holding up the fingers of the right hand. Respiratory data (time-locked to both image acquisition and stimulus presentation) was collected using a respiratory belt (PowerLab

16/35 and LabChart (ADInstruments; <http://www.adinstruments.com/>) and analysed using LabChart and custom python scripts.

Participants completed four runs, each consisting of randomised placebo, control and conditioning trials (Figure 9).

Figure 9 - Experimental Design



Participants inhaled either a low or high dose of nebulised capsaicin for 16s during six stimulus trials throughout a single run of functional brain image acquisition lasting 7 minutes and 12 seconds. All participants completed four scanning runs. Air was inhaled through a nasal cannula prior to each stimulus trial. A visual cue to participants indicated if

lidocaine or normal air would be delivered through the nasal cannula. However, participants only ever received normal air through the nasal cannula. Low doses of capsaicin preceded by placebo lidocaine were used to sustain the conditioned belief that the treatment was effective. High doses of capsaicin preceded by normal air constituted control trials. Placebo antitussive trials consisted of high doses of capsaicin preceded by placebo lidocaine. At the conclusion of each trial, participants were asked to indicate their rating of maximum urge-to-cough for the preceding stimulus using the fingers of the right hand (scale 0 to 10).

Statistical Methods

Behavioural analyses were carried out with PASW statistics (Release Version 18.0.0, SPSS, Inc., 2009, Chicago, IL, www.spss.com) using repeated measures ANOVA.

MRI analysis was performed using FEAT, Version 5.98, part of the FMRIB Software Library (FSL) (www.fmrib.ox.ac.uk/fsl/), using standard preprocessing and general linear modelling as described previously (see (Mazzone et al., 2011) for details). Briefly, preprocessing of EPI images consisted of realignment to correct for motion using a rigid body transformation, spatial smoothing with a 5mm full-width half-maximum Gaussian kernel, masking to remove non-brain tissue and high-pass temporal filtering with a cutoff of 0.01Hz. General linear modelling was carried out using

FILM with pre-whitening to account for local autocorrelation (Woolrich, Ripley, Brady, & Smith, 2001).

Respiratory data was low-pass filtered with a cutoff of 0.5Hz and custom python scripts were used to establish individual timing regressors for each capsaicin or nasal inhalation period based on respiratory phase. This allowed us to deliver stimuli at regular intervals and later reconstruct the exact time point at which exposure to the stimulus began. This is a departure from our previous methods and avoided the need to cue subjects to begin inspiration at particular times; removing unnecessary breath holds that are known to introduce design-correlated respiratory artefacts (Abbott et al., 2005). Participants were instructed to breath normally throughout the session and were not given any cues that inhalation periods were about to begin.

Timings for each trial were then retrospectively adjusted so as to begin immediately if the participant was already inhaling, or else at initiation of the next inhalation. Similarly, the end of the trial period was set to the conclusion of expiration. Respiration rates were examined for any potential differences between trial types and no significant differences were observed ($F(2,32)=1.34$, $p=0.275$; mean breaths per minute \pm S.D.; control = 13.9 ± 3.2 , conditioning = 13.9 ± 3.3 , placebo antitussive = 13.6 ± 3.1).

fMRI Analysis

fMRI analysis was performed using approaches previously described (Farrell et al., 2012; Mazzone et al., 2011). Regressors were constructed for each of the capsaicin inhalation and nasal inhalation conditions (placebo, control and conditioning) as well as the rating period. In a first level analysis these regressors and their temporal derivatives were convolved with a hemodynamic response function and used to generate a model for each run.

Additional regressors of no interest were included in the general linear model to account for any residual respiratory related noise as advocated by Birn (Birn et al., 2009). These consisted of six motion correction parameters, timecourses from cerebrospinal fluid (CSF) in the lateral ventricle and voxels of high standard deviation in the sagittal sinus, and mean global signal from non-activated areas of the brain (Andersson, 1997). Contrasts were performed for each of the main conditions, as well as the differences between control and placebo capsaicin inhalation blocks. A second level fixed effects analysis was used to amalgamate results from all 4 runs for each subject.

Regional brain activations were identified for control trials, placebo trials, and the contrasts of control greater than placebo and placebo greater than control trials. The association between participants' behaviour and regional signal changes was tested by analysing the shared variance between placebo effects (urge-to-cough ratings during control trials minus ratings during placebo trials) and the levels of activation identified by the contrast of placebo greater than control trials. For group analysis, individual T1 weighted images were transformed into a standard space based on the MNI152 (Montreal Neurological Institute) template. Mixed effects analysis was then performed using FLAME across subjects to generate a group result. All statistical maps were thresholded to include voxels with a Z-value >2.3 and a cluster probability of $p < 0.05$, corrected for multiple comparisons using Gaussian random field theory cluster-based correction as implemented in Feat (Worsley, Evans, Marrett, & Neelin, 1992).

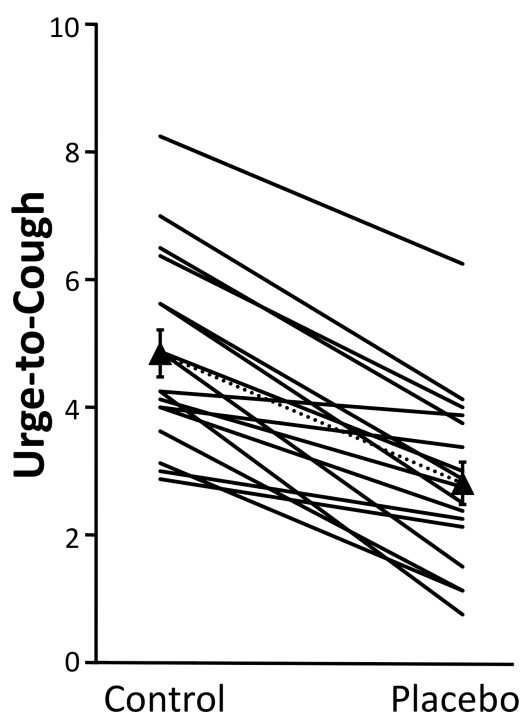
The relationship between placebo effects and activation for the contrast of placebo greater than control trials was examined with a region of interest (ROI) approach. An ROI was defined as those voxels in the dorsolateral prefrontal cortex that showed activation related to the difference between urge-to-cough ratings of placebo and control trials. Featquery was used to calculate percentage signal changes for the placebo greater than control contrast. Placebo effects on urge-to-cough ratings were ranked across the four scanning runs for each participant so that data could be amalgamated and used as independent variables in regression analyses to predict percentage signal changes from the regions of interest.

Results

Psychophysical Results

Repeated measures ANOVA of urge-to-cough ratings during control and placebo trials showed a significant main effect of condition ($F(1,16)=71.11$, $p < 0.001$). On average there was a 42% decrease in urge-to-cough ratings in placebo compared to control trials and a placebo antitussive effect was seen in all individuals (Figure 10).

Figure 10 – Urge-to-Cough Ratings During Control and Placebo



Participants' ratings of the urge-to-cough were averaged across the functional brain imaging runs for both control trials and placebo antitussive trials. The figure shows the pair of ratings from individual participants represented as unbroken lines. A downward slope to the right indicates a placebo-related decrease in the urge-to-cough. All participants showed placebo-related decreases, and the average effect across the group is represented by the filled triangles connected by an unbroken line. The variance bars on the triangles are standard errors of the mean.

The geometric means of low and high capsaicin doses were 0.39 μ M and 1.66 μ M, respectively. The high

stimuli were a dose increment less than the geometric mean of participants' C2 doses (3.91 μ M), which relates to increased levels of urge-to-cough and likelihood of coughing when participants inhaled capsaicin repeatedly compared to a single breath (see Table 2 for individual scanning doses and C2 thresholds). Titration of individual doses during preliminary trials successfully avoided any coughing events during scanning in all but one participant. Coughing during scanning in the one participant was managed by reducing the dose for placebo and control runs before further scans were acquired.

Table 2 - Individual Capsaicin Doses

Subject No	Low Dose (μ M)	High Dose (μ M)	C2 Dose (μ M)
1	0.25	1.95	7.81
2	0	0.98	0.98
3	0.25	0.98	3.91
4	0	0.98	1.95
5	0.25	1.95	3.91
6	0.735	3.91	3.91
7	0.49	1.95	1.95
8	0.25	0.98	0.49
9	0.06	1.465	1.95

10	0.49	1.95	3.91
11	1.95	5.86	3.91
12	0.25	0.98	15.63
13	0.98	1.465	3.91
14	0.25	0.98	1.95
15	0.49	1.465	31.25
16	1.465	5.86	*>62.5
17	0.25	0.98	1.95
Geometric Mean	**0.39	1.66	3.91

* C2 of participant exceeded doses used in experimental protocol

** Geometric mean calculated using non-zero values

Definition of abbreviations:

C2 – Dose of capsaicin that elicited 2 or more coughs on single maximal inhalation challenge.

Regional Brain Activation During Control Trials

Capsaicin inhalation during control trials was associated with widespread activation in a distributed urge-to-cough neural network, comparable to findings that we have previously reported (Farrell et al., 2012; Mazzone et al., 2007). Thus, activations in the cerebral cortex included loci in the precentral and postcentral gyri (primary motor and sensory cortices, respectively), the insula, mid cingulate cortex, and orbitofrontal cortices, and the supplementary motor area (Table 3). In subcortical regions, discrete activations were noted in the thalamus, midbrain, pons, medulla and cerebellum (Table 4).

Table 3 - Regional Brain Activations for Contrast Control > Baseline

Region	MNI Coordinates					Cluster	Z Score
	BA	Side	x	y	z		
Mid Cingulate Cortex	32	Right	4	18	42		5.01
	24	Left	-6	18	32	301	5.12
	24	Right	4	16	32		4.46
Medial Frontal Gyrus	6	Left	-4	30	32		4.21
Supplementary Motor Area	6		0	-4	60		3.31

Superior Frontal Gyrus	6	Left	-16	-4	68		3.98
	6	Right	26	2	66		3.94
	9	Left	-30	46	28		4.50
	9	Right	30	54	30	34	4.68
Middle Frontal Gyrus	6	Right	42	8	60		3.88
	46	Left	-36	40	26		4.53
	46	Right	46	38	26	314	4.76
	46	Right	38	32	22		4.60
	10	Left	-32	38	20	148	4.84
	10	Right	36	44	0		5.86
Orbitofrontal Cortex	11	Left	-44	44	-14	16	3.67
	11	Right	46	48	-16		4.37
Inferior Parietal Lobule	40	Right	52	-40	46		4.26
Cuneus	18		0	-88	28		4.15
Precentral Gyrus	6	Left	-54	-10	36		4.31
	6	Left	-42	-16	32		3.97
	6	Right	40	-14	32		3.70
	6	Left	-44	-12	22		3.64
	6	Right	40	-14	26	43	4.53
	6	Right	58	4	18	155	5.04
Postcentral Gyrus	2	Left	-66	-18	28		3.44
	1	Right	60	-16	22		3.98
Central Operculum		Left	-56	0	8	80	4.73
		Right	60	2	10		4.56
Superior Temporal Gyrus	22	Left	-56	8	-2		4.26
Insula	13	Left	-36	16	-2		5.16
	13	Right	36	20	0		6.09
	13	Left	-40	4	-10		5.24
	13	Right	38	4	-6		5.37

Definition of abbreviations:

BA - Brodmann Area

MNI Coordinates - The coordinates correspond to the Montreal Neuroscience Institute standard brain template where x values are distance in mm to the left (negative x values) or right (positive x values) from the anterior commissure, y represents mm distance anterior (positive) or posterior

(negative) from the anterior commissure and z is mm distance superior (positive) or inferior (negative) from the anterior commissure. The magnitudes of the peaks of clusters are represented by z statistics. The coordinates of the voxel with the highest level of activation within a cluster are listed.

Table 4 - Regional Subcortical Cerebellar and Brainstem Activations for Contrast Control > Baseline

Region	Side	MNI Coordinates			Cluster	Z Score
		x	y	z		
Globus Pallidus	Left	-14	0	-4		3.51
	Right	14	2	-6		3.24
Thalamus	Left	-16	-10	10		3.13
	Right	14	-12	4		3.50
Cerebellum						
Lobule VI	Left	-12	-66	-24	1908	5.22
	Right	10	-64	-20		5.74
	Left	-32	-60	-40		5.81
Dentate	Left	-20	-68	-26		6.33
	Right	22	-60	-28		5.88
Midbrain	Left	-10	-22	-6		3.23
	Right	14	-18	-10		3.22
Dorsal Pons	Left	-4	-36	-26		3.26
	Right	8	-36	-26	34	3.71
Rostral Medulla	Left	-6	-38	-50		4.75
	Right	4	-38	-48		4.82
Caudal Medulla		2	-42	-60	20	4.64

Definition of abbreviations: See Table 3 for explanations

Regional Brain Activation During Placebo Trials

In general, regions activated during placebo antitussive trials were similar to the network seen for control stimulus periods (Table 5).

Table 5 - Regional Brain Activations for Contrast Placebo > Baseline

Region	MNI Coordinates					Cluster	Z Score
	BA	Side	x	y	z		
Mid Cingulate Cortex	32	Left	-6	18	38	182	5.56
	32	Right	6	20	42		4.48
Superior Frontal Gyrus	9	Left	-30	48	28		3.80
Middle Frontal Gyrus	6	Right	40	10	60	20	4.40
	9	Right	34	52	34		4.50
	46	Right	48	38	26	98	5.42
	10	Left	-36	58	14		4.27
	46	Left	-44	50	10		4.40
	46	Right	46	54	8		4.86
	10	Left	-42	48	-8		4.55
	10	Right	46	50	-10		5.31
	10	Left	-42	54	-16	17	4.68
	10	Right	46	50	-16		5.43
Inferior Parietal Lobule	40	Left	-50	-48	54		4.56
	40	Right	58	-50	48		4.19
	40	Left	-48	-42	34	19	4.35
	40	Right	62	-46	38	23	4.04
Cuneus	18		0	-90	28		4.00
Precentral Gyrus	4	Left	-40	-18	30		3.84
	4	Right	40	-10	28		3.28

	6	Right	56	2	20		5.06
Central Operculum		Left	-56	0	8		4.56
		Right	60	2	12		4.56
Inferior Frontal Gyrus	47	Left	-28	22	-10		5.26
		Right	30	24	-8	380	4.75
Insula	13	Left	-32	4	4		4.36
	13	Right	36	0	-8	21	4.29
	13	Left	-34	18	-4	248	5.40
	13	Right	36	20	0		5.22
Cerebellum							
Vermis VI		Left	-2	-72	-14		5.38
Lobule VI		Left	-22	-68	-30	2427	5.98
		Right	24	-62	-28		5.63
		Left	-30	-58	-38		5.70
Dentate		Left	-34	-62	-48		5.45
Dorsal Pons		Left	-6	-34	-26		3.10
		Right	6	34	-22		3.12
		Left	-8	-38	-34		4.72
		Right	10	-40	-34		4.72
Rostral Medulla		Left	-6	-38	-48	132	5.24
		Right	6	-38	-48		4.60
Caudal Medulla		Left	-4	-42	56		4.89
		Right	4	-42	-56		4.48

Definition of abbreviations: See Table 3 for explanations

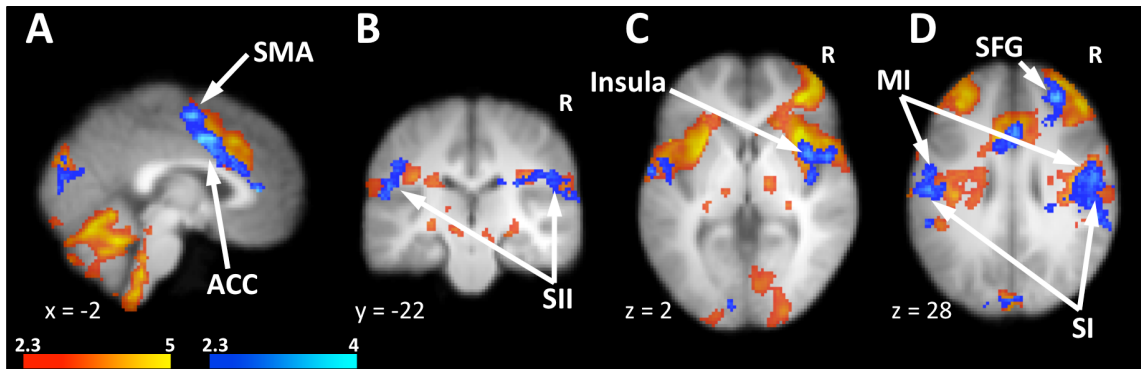
Mixed effects analysis showed significantly greater activation during control trials compared to placebo antitussive trials in regions including the primary and secondary somatosensory cortices, primary motor cortices, mid cingulate cortices and supplementary motor area ($p < 0.05$, cluster corrected)(Figure 11 and Table 6).

Table 6 - Regional Brain Activations for Contrast Control > Placebo

Region	MNI Coordinates					Cluster	Z Score
	BA	Side	x	y	z		
Mid Cingulate Cortex	24	Left	-2	2	42	215	3.88
	24	Right	4	22	24		3.70
Pregenua Cingulate Cortex	32	Left	-2	38	8	10	3.68
Superior Frontal Gyrus	6	Left	-18	-4	62	14	3.84
	6	Right	14	-8	64	52	3.62
	9	Right	30	44	28	141	3.67
Supplementary Motor Area	6		0	-4	58	48	3.95
Precentral Gyrus	6	Left	-52	-4	26		3.23
	6	Right	54	-6	28		3.32
Postcentral Gyrus	3	Left	-50	-18	36		3.48
	3	Right	52	-20	30		3.29
	3	Left	-56	-14	24	132	3.50
	43	Left	-60	-12	22		3.21
	43	Right	58	-8	20		3.10
Central Operculum		Left	-60	-10	12		3.26
		Right	54	2	8	138	3.77
Parietal Operculum		Left	-54	-22	22		3.25
		Right	50	-24	28		3.21
Cuneus	18	Right	4	-92	20		3.27
Superior Temporal Gyrus	22	Left	-54	8	-2	19	3.74
Insula	13	Right	40	8	2		3.41
	13	Right	42	-8	2		3.07

Definition of abbreviations: See Table 3 for explanations

Figure 11 – Regional Decreases in Brain Activation Following Placebo



Brain regions showing activation during control trials are rendered in red/yellow on an average of participants' structural brain images that were warped to the standard dimensions of the Montreal Neuroscience Institute template brain. A contrast was made between control and placebo antitussive trials to show regions where the level of activation was decreased after placebo lidocaine compared to control, the results of which have been rendered in blue on top of the structural image and control activations. **A.** A sagittal slice through the left hemisphere shows placebo antitussive-related decreases in capsaicin activation in the supplementary motor area (SMA) and anterior cingulate cortex (ACC). These placebo antitussive-related decreases also occurred in the right hemisphere (not shown). **B.** The secondary somatosensory cortex (SII) in the parietal operculum showed bilateral decreases in capsaicin activation during placebo antitussive trials. **C.** The insula (INS) was activated in both hemispheres during control stimulus periods, but showed placebo antitussive-related decreased activation exclusively in the right hemisphere. **D.** Control activations in the primary motor (MI) and somatosensory (SI) cortices were reduced bilaterally during placebo antitussive stimulus periods. A similar decrease was seen in the right superior frontal gyrus, but the same region in the left hemisphere did not show placebo antitussive-related decreases.

Conversely, there was greater activation in placebo antitussive trials versus control trials in several brain regions including, the dorsolateral prefrontal cortices in the middle frontal gyri, the precentral gyri, the inferior frontal gyri, the left inferior parietal lobule and the cerebellum ($p < 0.05$, cluster corrected)(Figure 12 and Table 7). The levels of increased activation during placebo antitussive compared to control trials in the right middle frontal gyrus were positively correlated with placebo-related changes in urge-to-cough ratings ($p < 0.05$, cluster corrected).

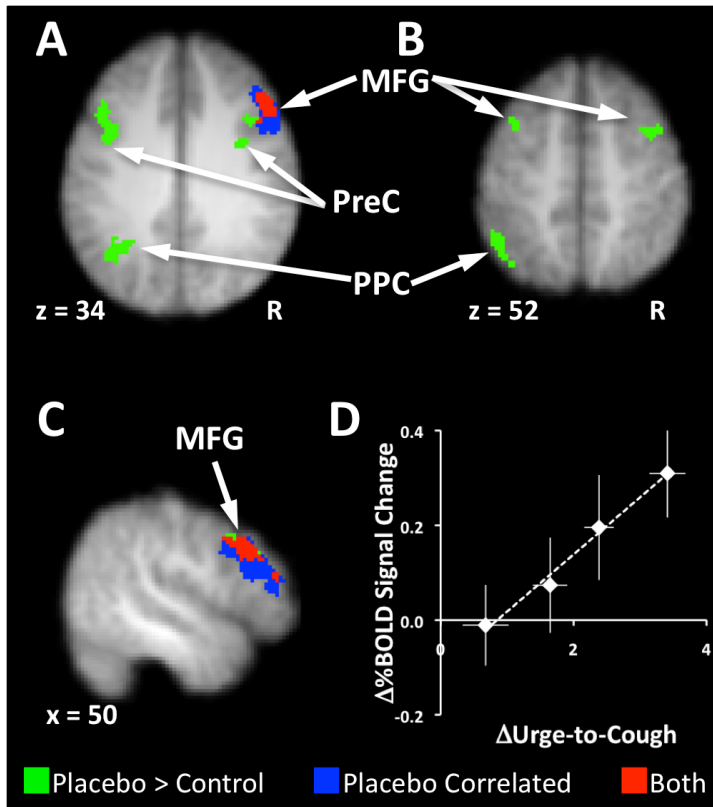
Table 7 - Regional Brain Activations for Contrast Placebo > Control

• Region	• BA	Side	MNI Coordinates			Z Score
			x	y	z	
Middle Frontal Gyrus	6	Left	-36	10	54	3.32
	6	Right	40	6	50	3.44
	9	Left	-40	20	36	3.35
	9	Right	46	26	34	*3.52
	9	Right	46	32	28	*3.19
Inferior Frontal Gyrus	45	Left	-40	22	22	3.75
	9	Left	-46	12	26	3.46
	9	Right	40	8	26	3.15
Precentral Gyrus	6	Left	-40	4	34	3.20
	6	Right	36	4	34	3.22
Inferior Parietal Lobule	40	Left	-50	-54	50	3.53
	40	Left	-34	-56	46	3.11
Middle Temporal Gyrus	39	Left	-32	-58	32	3.24
Cerebellum		Right	26	-88	-34	3.84

Definition of abbreviations: See Table 3 for explanations

** Clusters that showed levels of activation that were correlated with behavioural measures of placebo antitussive effects*

Figure 12 - Regional Increases in Brain Activation Following Placebo and Correlations with Behavioural Measures



Voxels shaded in green indicate regions where capsaicin activation was increased during placebo antitussive trials compared to control trials. Blue voxels denote regions where the size of the placebo antitussive increase in brain response was correlated with the size of the behavioural measures of placebo effects. Regions that showed placebo antitussive-related increases in capsaicin activations as well as correlations with the behavioural measures are shown in red. A. The posterior parietal cortex (PPC) in the left hemisphere and the precentral gyrus (PreC) bilaterally showed increased levels of placebo antitussive trial activation compared to control trials. The middle frontal gyrus (MFG) in the right hemisphere showed placebo antitussive-related levels of activation that correlated with the size of behavioural placebo effects measured with urge-to-cough ratings. B. Placebo antitussive trial-related increases in activation greater than control trials were seen bilaterally in the middle frontal gyri (MFG) in this axial slice 52mm above the anterior commissure. These symmetrical regions of activation did not show levels of activation that correlated with behavioural measures of the placebo antitussive effect. C. An extensive region of the right middle frontal gyrus (MFG) in the dorsolateral prefrontal cortex showed levels of placebo antitussive greater than control trial activation that was correlated with behavioural measures of placebo effects. D. The mean ratings of urge-to-cough by a participant for placebo antitussive trials during a scanning run were subtracted from the mean of the control trials in the same run to derive a behavioural measure of placebo effect (Δ Urge-to-Cough). Blood oxygen level-dependent (BOLD) signal changes for the contrast of placebo greater control trials ($\Delta\%$ BOLD Signal Change) were extracted for voxels showing correlation with the behavioural placebo effect. The relationship between behaviour and regional brain activation is represented in the scattergram where the four runs were ranked according to behavioural placebo effects from smallest to largest and then plotted against the mean BOLD signal changes for the corresponding runs.

Discussion

The brain plays an important role in both the processing of incoming sensory information arising from the airways and lungs and in generating motor outputs that contribute to the behavioural regulation of respiration. These central processes are not confined to the brainstem but rather involve all levels of the neuraxis (Davenport & Vovk, 2009; Mazzone et al., 2013). With respect to cough, information from the airways can be encoded into a conscious awareness of airway irritations leading to the generation of an urge-to-cough (Davenport et al., 2002), which may then facilitate behavioural or evoked coughing in order to help clear the airways (Hegland, Bolser, & Davenport, 2012). Components of this higher brain circuitry also comprise inhibitory mechanisms that can be consciously or subconsciously recruited to suppress cough neural processing in the brain (Mazzone et al., 2011). This suppression may lead to either a reduction in the encoding of

incoming sensory information, which would predictably lower the urge-to-cough, or a reduction in the outgoing motor commands that lead to a top down inhibition of coughing. Indeed the available evidence indicates that central inhibitory mechanisms are capable of an impressive level of cough suppression (Eccles, 2002; Hutchings et al., 1993), suggesting that opportunities may exist to harness or mimic these processes as novel therapeutic approaches to achieve cough suppression in disease.

In the present study we confirmed our previous findings (Leech et al., 2012) that the perceptual component of airway sensory irritation (urge-to-cough) is significantly modifiable by placebo antitussive treatments. Thus, the belief in a therapeutic is seemingly sufficient to reduce the encoding of urge-to-cough intensity in the brain. In support of this, the results from our functional brain imaging studies revealed that the magnitude of brain activations in a number of central loci involved in generating the urge-to-cough were significantly reduced by placebo antitussive administration. Furthermore, we showed that when participants believed that they were receiving an antitussive treatment, brain activity was increased in regions of the prefrontal and parietal cortices that may represent important components of the placebo suppression network (Benedetti, Mayberg, Wager, Stohler, & Zubieta, 2005; Krummenacher et al., 2010). These data represent the first insights into the neurobiological mechanisms that contribute to placebo antitussive responses and reinforce the notion that multiple central inhibitory mechanisms exist for modifying cough in humans.

Placebo antitussive suppression of the urge-to-cough

The urge-to-cough is an important component of coughing that serves to inform individuals of the presence of irritants in the airways. Studies in the laboratory have shown that common tussive challenges (e.g., capsaicin) can be used to evoke an urge-to-cough in a dose-dependent fashion (Davenport et al., 2002; Farrell et al., 2012). Furthermore, the urge-to-cough typically precedes the motor act of coughing, perhaps arguing that it plays a pivotal role in governing whether patients cough or not in response to sensory input from the airways (Davenport, 2008).

In the present study we noted a 42% reduction in the mean capsaicin-evoked urge-to-cough ratings following placebo antitussive administration. This response is highly comparable to our previous work, which was performed using an entirely separate cohort of participants (Leech et al., 2012). In both studies we employed a standard conditioning strategy to reinforce expectations that the placebo treatment was in fact an efficacious antitussive therapy. Using this strategy we have yet to

identify any participants who do not report a decrease in urge-to-cough ratings following placebo antitussive trials. These experimental data resemble data obtained from clinical trials that report a high rate of placebo responses to antitussive therapies (Eccles, 2002), adding support to our assertions that cough is particularly amenable to suggestive suppression. Indeed, cough may be more susceptible than pain to the effects of placebos, since placebo analgesia studies typically report that only around 50% of subjects experience a modest reduction in pain scores (Wager et al., 2004).

It is unclear at present why cough neural networks display such a high sensitivity to placebo antitussive suppression. It is worth noting that behavioural aspects of placebo suppression have not been studied in detail for cough. In this regard, we do not know whether the magnitude of placebo antitussive suppression varies depending on the magnitude of the initial stimulus. In other words, it is plausible that the placebo effect diminishes in participants when evoked cough approaches challenge thresholds that would elicit uncontrollable reflex coughing (and therefore very strong urge-to-cough sensations). Furthermore, it is simply not known whether patients with a cough disorder are equally sensitive to placebo antitussive suppression. Nevertheless, the size of the urge-to-cough reduction reported in our studies of healthy participants is not dissimilar to the magnitude of placebo contribution to cough suppression reported in many clinical trials of antitussive therapies, including in patients with chronic cough (Kuhn, Hendley, Adams, Clark, & Gwaltney, 1982; Lee et al., 2001; Parvez, Vaidya, Sakhardande, Subburaj, & Rajagopalan, 1996).

It has been speculated that decreased anxiety or attention may play a role in placebo analgesia (Flaten, Aslaksen, Lyby, & Bjorkedal, 2011). Indeed our paradigm shares some similarities with “threat of pain” studies (Ploghaus et al., 2001). Most notably, the preparatory cues used in our study could have primed emotional responses in anticipation of stimuli. However, it is difficult to make direct comparisons between the activation outcomes of the other studies and our own because threat of pain studies focus on adverse, anxiety-related responses, whereas our study investigated expectations of relief. Nevertheless, given that anxiety is known to influence cough (Van den Bergh et al., 2012), then reduction in anxiety may have played a role in producing the placebo antitussive effects observed in this study, and indeed could feasibly also contribute to the placebo effects seen in clinical trials (Watson et al., 2009).

This study used a conditioning strategy common to experimental placebo manipulations (Wager et al., 2004), where, rather than using an active drug, conditioning was achieved by means of pairing a visual cue with a reduced level of stimulus. As an initial investigation into the neural mechanisms underlying placebo in urge-to-cough, our design incorporated both enhanced expectation and

conditioning in order to maximize the size of the observed effect. Further investigation into the behavioural and neural responses to either conditioning or expectation alone may be instructive in elucidating the relative contributions of each to the behavioural effect, and also whether activation changes in similar cortical regions are involved. Although it is possible to design studies dissecting the relevant contributions of these mechanisms towards experimental placebo effects, it may be more difficult to conceptually separate them in clinical settings. For example, prior experience using ineffective antitussives may lead not only to negative expectations surrounding treatment, but may indeed be thought of as a form of conditioning, whereby patients are conditioned to associate treatment with a lack of effect (Colloca & Benedetti, 2006).

Regional brain responses during capsaicin inhalation and the effect of placebo

The urge-to-cough relies on subcortical and cortical processing of incoming sensory information from the airways (Farrell et al., 2012; Mazzone et al., 2007; Wheeler-Hegland, Pitts, & Davenport, 2010). We have previously reported that subcomponents of this broader urge-to-cough network can be delineated that show activations encoding for the sensory discriminative, cognitive and motor aspects of airways irritation. For example, based on differential responses to varying levels of tussive stimuli, we have shown that distinct parts of the network activated by capsaicin inhalation are responsible for grading stimulus intensity (e.g., anterior insula cortex), determining urge-to-cough intensity (e.g., primary sensory cortex) and processing spatial or higher order processes such as those related to attention and motivation (e.g., the inferior parietal lobule and prefrontal cortex)(Mazzone et al., 2013). We have also identified discrete regions within the right inferior frontal gyrus and anterior insula as forming part of an endogenous cough suppression network that can be voluntarily recruited to reduce motor outputs driven by the urge-to-cough (Mazzone et al., 2011) and the mid cingulate cortex as a key hub area that may help integrate all of these functions of the urge-to-cough network (Farrell et al., 2012).

The results of the present study suggest that the different components of the urge-to-cough network do not respond uniformly in response to placebo antitussive administration. For example, capsaicin-related activations following placebo antitussive administration were significantly reduced in several sensory and motor regions, including the insula, anterior cingulate cortex, primary somatosensory and motor cortices and the supplementary motor area. By contrast, placebo increased the activity in the middle frontal gyri (specifically in the region containing the dorsolateral prefrontal cortex, Brodmann area 9) and in the inferior parietal lobule and precentral gyrus. These findings might reflect regional responses that are responsible for the behavioural

outcomes following placebo antitussive administration. Thus, the reduction in urge-to-cough ratings reported by participants following placebo presumably reflect the relatively lower amount of activation seen in the urge-to-cough sensorimotor brain regions. Furthermore, these reductions may be related to the heightened activity noted elsewhere in the urge-to-cough network. Indeed, placebo analgesia studies similarly report elevated brain activity in the dorsolateral prefrontal cortex and inferior parietal lobule, suggesting that these regions may play an important role in regulating sensorimotor responses in the brain (Amanzio, Benedetti, Porro, Palermo, & Cauda, 2013).

Consistent with this, the magnitude of the activation in the right dorsolateral prefrontal cortex in the present study was significantly correlated with the magnitude of the reported placebo antitussive effect. Alternatively, regions including the right ventral inferior frontal gyrus, neighbouring anterior insula and the more anterior extent of mid cingulate activation, regions which have been implicated in cough and breathing suppression (Mazzone et al., 2011; McKay, Adams, Frackowiak, & Corfield, 2008), did not show differences between placebo and control trials.

These results point to similarities in the mechanism of action of placebos between pain and urge-to-cough. The decreases in activation in capsaicin inhalation networks can be compared to results from placebo analgesia studies, where the network of pain-related brain regions shows decreased activation in response to painful stimuli following placebo (Lu et al., 2010; Lui et al., 2010; Wager et al., 2004). Studies of placebo effects in pain have also shown increased activation in the dorsolateral prefrontal cortex (Wager et al., 2004; Watson et al., 2009) in the placebo condition. Furthermore, placebo analgesia is abolished when transcranial magnetic stimulation is used to block neural transmission in this region (Krummenacher et al., 2010).

Interestingly, there were no statistically significant decreases in brain activity noted in thalamic or subcortical structures when responses following placebo antitussive administration were compared to no-treatment responses. This might suggest that placebo antitussive circuits function by selectively reducing cortical processing of incoming sensory information. However, the data might also reflect the scanning parameters employed, which were optimised for whole brain imaging rather than discrete imaging of subcortical and lower brain structures. Consistent with this, several thalamic and brainstem regions displayed responses approaching statistical significance following placebo antitussive administration, which may be better resolved using an acquisition protocol optimised for this purpose.

Concluding Remarks

Placebo antitussives have a strong influence on coughing and the urge-to-cough. The outcomes of this study have shown that the substantial reductions in urge-to-cough after placebo are matched by a parallel decrease in brain responses to capsaicin inhalation in regions coding sensation and processing motor outputs. This confluence of behaviour and brain activity points toward an active inhibitory process that is likely mediated by prefrontal cortex, parietal cortex and the cerebellum. Of those regions implicated in the placebo antitussive effect, activity in the right dorsolateral prefrontal cortex appears to have a special significance and constitutes a promising target for future studies. The study of higher brain mechanisms in cough has the potential to expand our understanding of a number of clinical conditions such as chronic idiopathic cough or chronic cough associated with COPD. In addition, it is highly likely that these mechanisms are involved in excessive cough syndromes with a psychological or behavioural component including psychogenic or habit cough, as well as disorders of abnormal cough down regulation or suppression such as Lady Windermere syndrome (Davenport, 2008; Van den Bergh et al., 2012; Weinberger, 2012). As these disorders are particularly difficult to treat, understanding the neurological basis of placebo suppression of urge-to-cough may be a promising avenue for developing or evaluating tools such as biofeedback, cognitive behavioural therapy or perhaps other behavioural therapy (e.g. speech therapy) that aim to establish a normal cough reflex. Further research may also prove useful for developing novel CNS drug targets.

Chapter 5 - Brainstem Activity Associated with Placebo Suppression of the Urge-to-Cough

Introduction

Administration of a placebo decreases the behavioural response to tussive stimuli, as well as causing a reduction in activation in urge-to-cough related brain regions such as somatosensory cortex. The placebo effect in urge-to-cough appears to be mediated by inhibitory processes involving parts of the prefrontal and parietal cortices. The right dorsolateral prefrontal cortex in particular is correlated with the strength of the observed effect. It is, however, unclear whether this inhibition is caused by a reduction in the transmission of urge-to-cough sensory information from the periphery to the cortex by descending inhibition of brainstem respiratory centres, or whether it is due to purely cortical inhibitory mechanisms. If inhibition of brainstem regions is implicated in producing placebo reduction of the urge-to-cough, then changes in processing in these pathways could feasibly be implicated in the excessive urge-to-cough seen in patients with chronic cough disorders.

The sensation of urge-to-cough is initiated by irritation in the airways activating peripheral sensory nerve receptors that are sensitive to either mechanical or chemical stimulation. Vagal afferent nerves convey these signals to the brainstem, where information is integrated before being relayed to the cortex. A distributed network of cortical regions further processes this information, incorporating psychological and social variables such as emotional state and social context. This process ultimately results in either suppression of the urge-to-cough, recognition of the urge-to-cough without motor output, or activation of the cough motor pattern generator in the brainstem, leading to reflex cough.

These vagal afferent nerves from the airways first synapse with second order neurons in the nTS. The region of the nTS in receipt of airway afferents is the caudal medial part of the nucleus rostral to obex (Canning & Mori, 2010). As the first CNS point of synapse where sensory input from the airways could be modulated, the nTS has been proposed as the most likely site for sensory integration and gating of the cough motor response (Bonham, Sekizawa, Chen, & Joad, 2006). Given the nTS receives peripheral sensory information, as well as thalamic and cortical input, it presents a likely candidate region for cortically mediated placebo suppression of the urge-to-cough.

Information from the airways is first integrated via local neuronal assemblies in the nTS, and the resulting signals are then relayed to neurons in the reticular formation, the parabrachial complex, particularly the Kolliker-Fuse nucleus (Torvik, 1956), and regions of the ventrolateral medulla that are related to respiratory control (Shannon, Baekey, Morris, & Lindsey, 1998). Additionally the nTS has projections to the hypothalamus, thalamus and amygdala (Canning, 2007; Mazzone & Canning, 2002; Ohi et al., 2005).

The nTS also receives direct projections from insular cortex, hypothalamus, amygdala, medial prefrontal cortex and lateral prefrontal cortex (Otake & Nakamura, 2003; Van Der Kooy, McGinty, Koda, Gerfen, & Bloom, 1982; Vanderkooy, Koda, McGinty, Gerfen, & Bloom, 1984), making it an ideal candidate region for top-down placebo related inhibition. Notably, these connections involve the commissural, or most caudal part of the nTS that receives inputs from airways afferents. Inhibition at this first synaptic level of sensory integration in the brainstem would cause decreased activation in all downstream regions of cough sensory processing, as was seen in our previous whole brain MRI study.

A model of the central reflex pathway regulating cough has been proposed (Bolser & Davenport, 2002; Shannon et al., 1998) that suggests sensory afferents converge on a putative gating mechanism via nTS relay neurons. Gated signals then pass to the central cough pattern generator. This proposed gating mechanism presents one possible point of placebo inhibition. Short-term neuronal plasticity in the nTS may contribute to heightened responses to tussive stimuli, or to the suppression of cough (Bonham et al., 2006; Bonham, Sekizawa, & Joad, 2004). Long-term plasticity in these neurons could also feasibly be responsible for the responses to normally innocuous stimuli seen in chronic cough patients (Bonham et al., 2006)

Newer studies using recombinant viral tracing have also shown that afferent neurons from the trachea can additionally be traced to the trigeminal and paratrigeminal nuclei in the medulla (McGovern et al., 2012). As there is evidence that these neurons relay sensory information to the thalamus via trigeminothalamic tracts (Cechetti & Saper, 1987), this represents an alternative pathway where inhibition could occur. Using anterograde viral tracing from tracheal afferent neurons, cells were labelled initially in the nTS and trigeminal/paratrigeminal nuclei. Secondary labelling occurred in the pons (lateral and medial parabrachial nuclei), thalamus, hypothalamus and amygdala, before being labelled in higher cortical regions including somatosensory, orbital, insula and cingulate cortices (McGovern et al., 2012).

Most of what is known about the brainstem circuitry that produces an urge-to-cough comes from animal models and has not yet been studied in humans. Thus, in addition to the brainstem nuclei already mentioned, other nuclei involved in respiratory and cough control could possibly play a role in the sensory perception of urge-to-cough. Reflex cough is produced by a brainstem respiratory network normally involved in regulating breathing (Shannon et al., 2004). This widespread network includes neurons in the ventrolateral medulla (including the Bötzing and pre-Bötzing complexes) that are normally responsible for respiratory pattern generation, the raphe nuclei in the caudal medulla and lateral tegmental field, as well as pontine respiratory centres and the cerebellum (Bolser & Davenport, 2002). The pontine respiratory group in the rostral dorsal lateral pons (including the Kölliker-Fuse nucleus) is also involved in maintaining a normal breathing pattern, and appears to be essential for producing reflex cough (Poliaček et al., 2004).

In this study our aim was to determine whether placebo inhibition of urge-to-cough occurs due to cortico-cortical inhibition, or whether descending inhibition of subcortical regions is also involved. If the placebo effect causes inhibition at a subcortical level, we would expect to see less activation in brainstem nuclei involved in relaying sensory information to the cortex. If on the other hand the placebo effect is mediated by purely cortical mechanisms, we would expect to see similar patterns of activation in brainstem regions during both placebo and no treatment (i.e. less activation in the cortex, given the same input from subcortical regions). This information could assist in assessing and targeting therapeutic interventions in chronic cough patients aimed at reducing the sensation of urge-to-cough without impairing reflex cough.

Methods

Participants

16 healthy, non-smoking subjects with no history of respiratory disorders participated in the experiment (6 male, mean age \pm s.d. = 24.25 years \pm 4.78 years). This study was approved by the Melbourne Health Human Research Ethics Committee (2013.078). In line with best practise in the use of deception in research, subjects were fully debriefed at the end of the session, both verbally and in writing. Participants were also given the opportunity to withdraw their data from the study if they so wished. None of the participants chose to use this option.

Capsaicin threshold testing

Individual perceptual and cough thresholds were tested with single maximal capacity inhalations of nebulised capsaicin in doubling doses ranging from 0.12 to 62.5 μ M. Following each inhalation participants were instructed to rate their urge-to-cough on a modified Borg scale ranging from zero (no urge) to ten (maximum urge). The urge-to-cough threshold (Cu) was defined as the first instance where participants reported an urge-to-cough, while the cough threshold (C2) was the first dose that provoked two or more coughs. In the subsequent fMRI session the high dose used was the maximum concentration of capsaicin that could be tolerated without causing reflex cough. The low dose was one quarter of this concentration. Doses were adjusted as necessary if participants showed sensitisation or habituation across the course of the session. The individual low and high doses reported are the mean doses received by each participant during the session.

Image Acquisition

Images were acquired with a 3T Siemens Trio at the Royal Children's Hospital in Parkville, Melbourne, Australia. Structural images consisted of sagittal T1-weighted whole brain images (TR=1900 ms, TE=2.59ms, 192 slices, 0.9mm thickness, 0.8x0.8mm in-plane resolution). Functional runs were collected using a partial FOV (See Chapter 2 for more details) with a gradient-echo echo-planar parallel imaging sequence (GRAPPA, PAT factor=3, reference lines=36, TR=1790ms, TE=30ms, 27 slices, 2.5mm thickness, 1.88mm in-plane resolution, flip angle=70 degrees). Three whole brain EPI images with the same resolution as the functional runs were also collected. The mean of these images was used for an initial registration of the partial FOV images to the whole brain.

Conditioning

Conditioning was carried out as per the previous experiment. Briefly, each participant underwent two conditioning runs, with each run consisting of three no-treatment trials and three lidocaine trials. Participants were informed that they would receive the same capsaicin dose in every trial, preceded by either lidocaine or no-treatment. In fact, participants received either no-treatment followed by a moderate dose of capsaicin, or placebo followed by a low dose of capsaicin.

Identification of Brainstem Regions Involved in the Placebo Effect on Urge-to-Cough

Participants completed three fMRI runs, each consisting of three presentations each of control (no-treatment followed by high dose capsaicin), conditioning (treatment followed by low dose

capsaicin) and placebo (treatment followed by high dose capsaicin), presented in a randomised order.

Participants were informed via a projector running PsychoPy software (Peirce, 2008) at the beginning of the lidocaine or control nasal inhalation periods, during capsaicin inhalation, and when they were required to rate their urge-to-cough. Control trials were differentiated from placebo and conditioning trials by the use of red and green text, respectively. At all other times participants were instructed to remain focused on a fixation cross that matched the colour of the current trial type. Participants were thus constantly aware of which trial they were currently completing.

Statistical Analysis

Psychophysical analysis was carried out using ezANOVA software (<http://www.mccauslandcenter.sc.edu/mricro/ezanova/>). fMRI analysis was carried out using the FMRIB suite of software tools (www.fmrib.ox.ac.uk/fsl/). Preprocessing of functional EPI data consisted of motion correction with McFlirt, spatial smoothing with a 4mm full-width half-maximum Gaussian kernel and high pass filtering with a 100s cutoff.

Respiratory data was low-pass filtered with a cutoff of 0.5Hz and LabChart (ADInstruments; <http://www.adinstruments.com/>) was used to determine the timing of maximal inhalation and exhalation. Custom python scripts were then used to establish individual timing regressors for each capsaicin or nasal inhalation period based on respiratory phase (See Chapter 2 for more detail).

Registration

Functional EPI data was first linearly registered to the mean whole brain EPI using FLIRT with three degrees of freedom. An initial transformation linearly registered the whole brain EPI to each subject's anatomical T1 scan using boundary-based registration (BBR)(Greve & Fischl, 2009), then FNIRT (Andersson, Jenkinson, & Smith, 2010) was used to non-linearly register the individual T1 images to a standard 1mm brain template (MNI152) in order to preserve spatial resolution.

General Linear Modelling

General linear modeling was carried out using FILM with pre-whitening to account for local autocorrelation (Woolrich et al., 2001). Nuisance regressors included 6 motion realignment parameters and any volumes where excessive motion occurred as implemented in

FSLMotionOutliers. Additional regressors of no interest included to account for physiological noise consisted of motion corrected but otherwise unprocessed timecourses from a voxel of high standard deviation in the sagittal sinus, voxels located in the lateral ventricles and a voxel of high standard deviation in each carotid artery.

Regressors were also created for each condition of interest: the period of capsaicin inhalation following control, placebo or conditioning; the period of “lidocaine” inhalation during control, placebo or conditioning; and the period where subjects were asked to rate their urge-to-cough. Regressors and their temporal derivatives were convolved with a canonical HRF. Contrasts were created for each of these regressors as well as the differences between control and placebo capsaicin inhalation periods. The resulting statistical maps covering the entire FOV were thresholded at $p < 0.05$ corrected for multiple comparisons using FWE, in order to compare activation in cortical regions to those from the previous study (Chapter 4).

As the brainstem regions involved in urge-to-cough have not been sufficiently well localised in humans to define a priori regions for statistical small volume correction, statistical maps for the relevant contrasts were first masked with an inclusive mask consisting of brainstem areas activated during all capsaicin inhalation periods obtained from the previous whole brain study (Leech, Farrell, & Mazzone, 2013)(Chapter 4). Masked statistical maps were then thresholded using permutation methods as implemented in Randomise (Winkler, Ridgway, Webster, Smith, & Nichols, 2014). Individual COPE images from the general linear model were fed into a separate Randomise analysis with 5000 permutations. Results were thresholded at $p > 0.05$, corrected for multiple comparisons using FWE, with a cluster extent of greater than 10 voxels. An analysis of correlation with behavioural measures added an additional variable consisting of the average change in urge-to-cough ratings (percent change in ratings between placebo and control trials) for each participant (demeaned over all participants) as a measure of the strength of placebo effect.

Results

Psychophysical Results

Repeated measures ANOVA of urge-to-cough ratings during control and placebo trials showed a significant main effect of condition (i.e. control versus placebo) ($F(1,15)=22.10$, $p < 0.005$). There was an average decrease in urge-to-cough ratings of 27% in placebo compared to control trials. The geometric means of low and high capsaicin doses used during the scanning procedure were $0.40\mu\text{M}$ and $1.83\mu\text{M}$, respectively. The high stimuli were, in all but one participant, equal to or lower than

the individual's C2, reflecting both the increased likelihood of coughing when participants are exposed to continuously inhaled capsaicin compared to single maximum capacity inhalation, and the experimental procedures that aimed to minimise coughing during the MRI scan by reducing the capsaicin dose if participants began to cough. Conversely, the relationship of the low dose to Cu was much more variable, with four participants having a low dose equal to their Cu, four participants having a low dose higher than their Cu, and the remaining eight participants having a low dose below their Cu. This is likely to reflect the difficulty of perception at near threshold doses of stimuli, as similar to the high dose, experimental procedures were followed where the low dose was increased if participants began to consistently rate the stimuli as zero. See Table 8 for individual thresholds and doses used during scanning.

Table 8 - Individual Capsaicin Doses

Subject No	Average Low Dose (μM)	Average High Dose (μM)	Cu Dose (μM)	C2 Dose (μM)
1	0.33	1.31	0.49	3.91
2	0.12	0.74	0.25	31.25
3	0.49	1.95	0.49	1.95
4	0.49	1.63	0.25	31.25
5	0.25	1.95	0.25	1.95
6	0.98	5.86	0.49	7.81
7	0.71	2.85	0.49	7.81
8	0.49	1.95	0.49	7.81
9	0.25	1.47	0.25	7.81
10	0.49	1.95	1.95	3.91
11	0.09	0.82	0.49	1.95
12	0.20	0.57	0.25	0.49
13	0.12	0.49	0.25	1.95
14	0.25	1.11	0.98	3.91
15	4.34	18.25	1.95	*>62.5
16	0.98	3.91	3.91	32.50
Geometric Mean	0.40	1.83	0.54	**6.04

* C2 of participant exceeded doses used in experimental protocol

** C2 of 62.5 μ M was used in calculating geometric mean for Subject 15

Confirmation of Placebo Related Changes in Activation in Expected Cortical Regions

Whole brain regions showing activation were restricted to the FOV acquired during functional runs, optimised for the brainstem. While this FOV did not cover many of the regions investigated in the whole brain study (for instance the DLPFC), the activations for the contrast control>placebo showed similar differences in activation to those seen in the whole brain study e.g. somatosensory and premotor cortices (Table 9)(See Chapter 2 for FOV information). No significant differences in activation were seen for the contrast placebo>control. This was the expected result, as the FOV did not cover the regions implicated in placebo suppression of urge-to-cough (PPC and DLPFC, see Chapter 4).

Table 9 - Regional Brain Activations for Contrast Control > Placebo

Region	BA	Side	MNI Coordinates			Cluster Size (Voxels)
			x	y	z	
Mid Cingulate Cortex	32	Left	-5	-42	5	1132
	32	Right	0	-37	38	806
Superior Frontal Gyrus	9	Left	-5	-3	71	765
Middle Frontal Gyrus	6	Right	-49	-54	-39	531
	9	Right	2	-59	-36	529
	46	Right	-53	-9	36	517
	10	Left	-60	-31	18	454
	46	Left	-58	-22	24	450

Definition of abbreviations:

BA - Brodmann Area

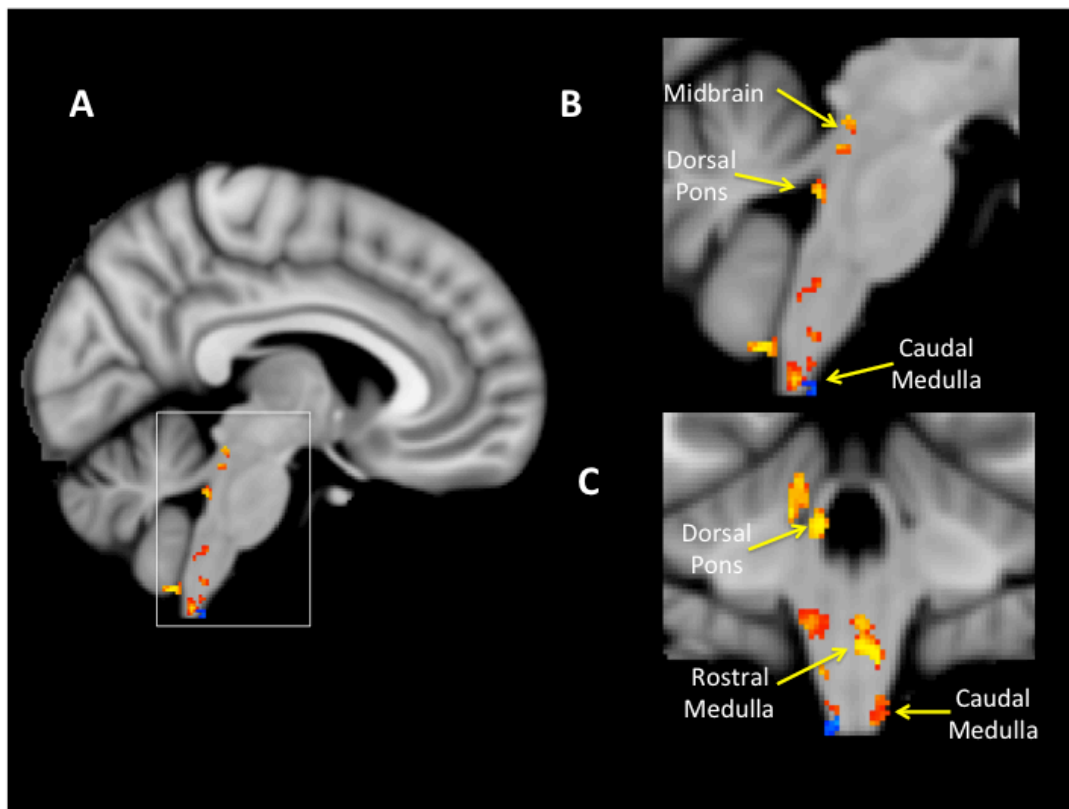
MNI Coordinates - The coordinates correspond to the Montreal Neuroscience Institute standard brain template where x values are distance in mm to the left (negative x values) or right (positive x values) from the anterior commissure, y represents mm distance anterior (positive) or posterior (negative) from the anterior commissure and z is mm distance superior (positive) or inferior

(negative) from the anterior commissure. The coordinates of voxels with the highest level of activation within a cluster are listed.

Decreased Brainstem Activation During Capsaicin Inhalation Following Placebo

Significant activations for the contrasts control > baseline and placebo > baseline were seen in a range of regions in the medulla and pons. The magnitude and extent of significant activation was greater in the control condition than in the placebo condition (Figure 13).

Figure 13 - Capsaicin Related Brainstem Activation Following Placebo and Control

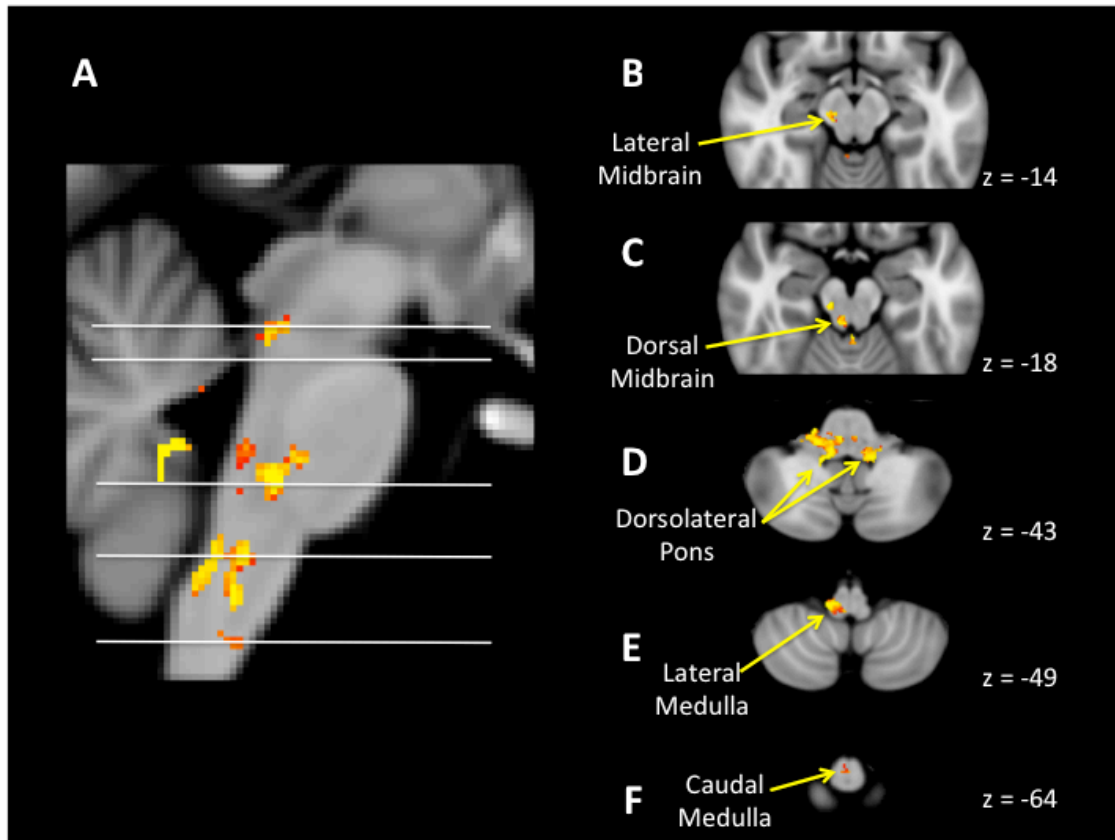


Capsaicin related activation during control (red) and placebo (blue). Shown at visualisation threshold of $p < 0.005$, uncorrected. Images are displayed in radiological convention (right side of the image shows the left side of the brain)

A) Sagittal slice showing brainstem region of interest. B) Sagittal brainstem slice showing control>baseline activation in midbrain, medulla, cerebellum and pons; and placebo>baseline activation in caudal medulla. C) Coronal slice showing bilateral medullary and pontine activation in contrast control>baseline and placebo>baseline activation in caudal medulla.

Mixed effects analysis of the contrast control > placebo showed significantly less activation following placebo in a subset of these regions. These included a number of respiratory-related pontine and medullary structures (Table 10 and Figure 14).

Figure 14 – Regional Decreases in Brainstem Activation Following Placebo



A) Sagittal cross-sections showing brainstem regions with significantly decreased capsaicin induced activation following placebo. Shown at a visualisation threshold of $p < 0.005$, uncorrected. Activation maps are overlaid on standard MNI brain template. Images displayed in radiological convention (right side of brain is displayed on the left of the image). Lines indicate level of respective axial cross-sections. B) Axial cross-sections showing pontine and medullary activation.

Correlation of Regional Placebo-Induced Signal Reduction with Behavioural Measures

The decrease in activation following placebo was also significantly correlated with the strength of the placebo effect in individual subjects in the left dorsolateral rostral pons (Table 10 and Figure 15). Regions with the largest signal decrease following placebo were correlated with stronger behavioural change. No correlations were seen with the opposite contrast (placebo > control).

Table 10 – Brainstem Activations for Contrast Control > Placebo

Control >Placebo					
Regions Activated	MNI Coordinates (COG)			Cluster p value	Cluster Size (Voxels)
	x	y	z		
Caudal Lateral Pons	9	-39	-46	0.003	402
Rostral Ventrolateral Medulla					
Rostral Dorsolateral Medulla					
Right Dorsolateral Caudal Pons	21	-33	-41	0.013	111
Right Dorsolateral Rostral Pons	10	-38	-25	0.03	15
Left Dorsolateral Caudal Pons	-12	-44	-44	0.024	13
Caudal Medulla	0	-42	-64	0.002	15

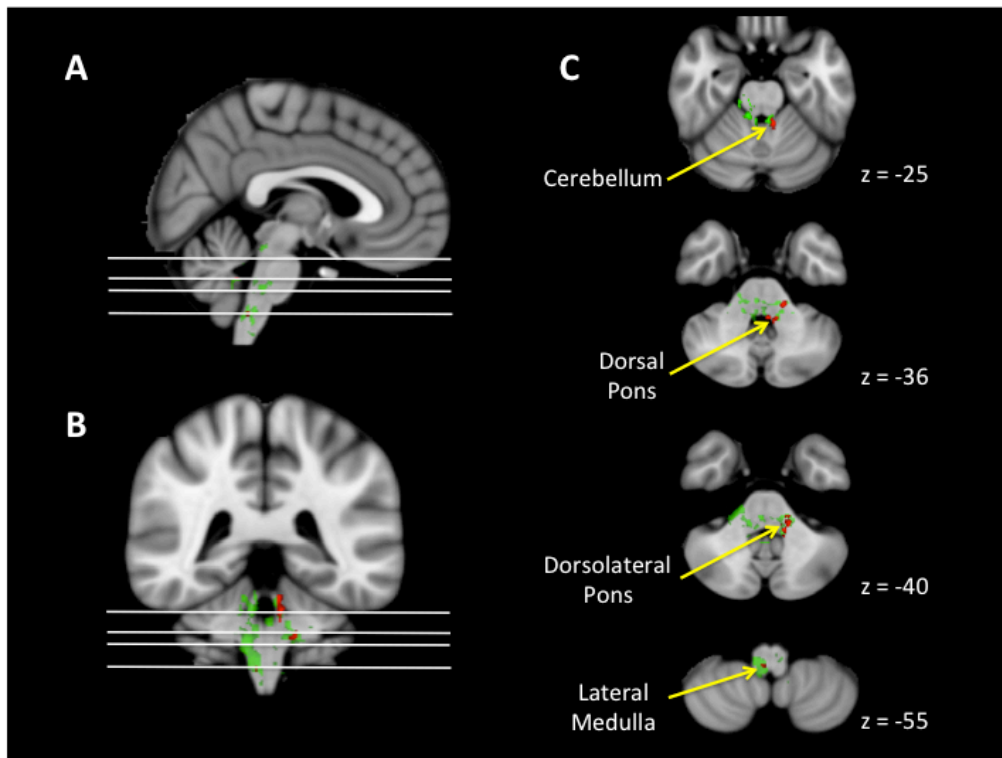
Activation Correlated with Urge-to-Cough Ratings

Regions Activated	MNI Coordinates (COG)			Cluster p value	Cluster Size (Voxels)
	x	y	z		
Left Dorsolateral Rostral Pons	-8	-43	-29	0.016	35

Definition of abbreviations: See Table 9 for explanations.

Clusters significant at $p < 0.05$, corrected (FWE) and extent of > 10 voxels. MNI co-ordinates correspond to centre of gravity (COG) of clusters.

Figure 15 - Regions where Decreased Activation following Placebo is Correlated with Behavioural Ratings



A) Sagittal cross-section and B) coronal cross-section showing left dorsolateral rostral brainstem regions (in red) where the strength of the placebo effect (difference between ratings in control trials and ratings in placebo trials for each individual) was correlated with the contrast control>placebo. Activation in control>placebo contrast is shown in the background in green. Shown at visualisation threshold of $p < 0.005$, uncorrected. Activation maps are overlaid on a standard MNI brain template. Images displayed in radiological convention (right side of brain is displayed on the left of the image). Lines indicate level of respective axial cross-sections. C) Axial cross-sections.

Discussion

Summary

The sensation of airway irritation termed the urge-to-cough is an important aspect of clinical respiratory pathophysiology (Davenport, 2009). The neural pathways involved in regulating sensory processing of afferent information from the airways have not yet been investigated in detail in humans. This study provides evidence that this processing can be inhibited even at the earliest stages of afferent input to the CNS.

This is the first indication that placebo can cause descending inhibition of sensory processing of afferent input from the airways at the level of the brainstem. These regulatory regions in the pons and medulla may have potential as targets in developing future treatments for chronic cough disorders, or for assessing the efficacy of potential antitussive therapies.

The administration of a placebo affected both cortical and subcortical processing of the urge-to-cough. Confirming the results of the previous study (Leech et al., 2013), placebo treatment was associated with significant decreases in capsaicin-related activation in a distributed network of cortical regions normally involved in sensing airway irritation. Furthermore, this decrease in activation was also apparent in respiratory-related subcortical regions, implying that simply the belief and expectation of receiving a treatment can cause inhibition at the first central sites where sensory information from the airways is integrated.

Placebo Suppression of the Urge-to-Cough

Unlike the previous studies, we did not see a placebo related decrease in urge-to-cough ratings in all participants. 14 out of 16 participants rated their urge-to-cough as lower on average following placebo. The mean difference in ratings between placebo and control conditions for all participants was still highly significant and again reflects a high rate of placebo response when compared to placebo effects in other systems (Benedetti, 2008; Hróbjartsson & Gøtzsche, 2004b).

Similar to the previous studies, high doses were on average one quarter of the C2 dose, however this did vary widely between subjects. This is likely to reflect the difference between single maximal inhalation challenges used to measure C2, and the experimental protocol that involved 16 second non-maximal inhalation periods. Similarly, the low dose was occasionally below the Cu in some individuals, reflecting the greater sensitivity to near perceptual threshold during continuous inhalation periods.

Brainstem Activity Associated with Placebo Suppression of the Urge-to-Cough

There was a significant reduction in capsaicin-related signals following placebo in widespread regions of the pons and medulla that are presumably related to relaying sensory information from the airways. During control trials, there was significant widespread bilateral activation in the medulla and pons, while during placebo trials this activation was limited to a circumscribed region in the right caudal medulla. The spatial pattern of capsaicin-induced activation seen in placebo trials was however similar to control trials at a lower statistical threshold, indicating that while signal change associated with neural activity in the placebo condition was not abolished, it was greatly reduced.

As there were no areas that showed significantly increased activation following placebo treatment, it is not possible to define precisely where this inhibition occurs. However, the widespread decrease in BOLD signals throughout the pons and rostral medulla suggests that inhibition is occurring at the level of the caudal medulla. Given the evidence pointing to the caudal nTS as a potential site for modulation of incoming sensory signals from the airways (Boscan et al., 2002; Canning & Mori, 2010), and the fact that the nTS receives input from cortical regions including the prefrontal cortex (Otake & Nakamura, 2003; Van Der Kooy et al., 1982; Vanderkooy et al., 1984), makes the nTS a likely candidate region for the cortico-medullary descending inhibition observed in this study. A region where activation strength and behavioural measures were correlated was also seen in the rostral dorsolateral pons, at the level of the pontine respiratory group, indicating that this region may also be involved in orchestrating the placebo response.

The limited amount of information about functional brainstem anatomy in humans, as well as issues of registration, precludes definitive identification of specific regions of activation. Variation in brainstem orientation and size between individuals makes registration to a group template challenging, and available tools are not optimised for the brainstem (Napadow et al., 2006). These results must be considered preliminary until more detailed studies of the functional anatomy of respiratory centres have been carried out. Future studies of cough sensory pathways in the brainstem using high-field MRI may lead to improved localisation of specific nuclei.

Similarity to Placebo Analgesia

This study points to similarities in the mechanisms of placebo suppression of the urge-to-cough and placebo analgesia. Studies have shown decreased pain-related activation following placebo in spinal

cord nuclei (Eippert et al., 2009a; Eippert et al., 2009b). Considering that similar cortical regions are recruited during placebo analgesia and placebo inhibition of urge-to-cough, it would appear that an analagous process of descending inhibition occurs, involving DLPFC and PPC and affecting activity even at the earliest level of central processing. Although this does not rule out the possibility of cortico-cortical inhibition also playing a role in placebo suppression of urge-to-cough, it does indicate that, at least experimentally, it is possible to suppress processing of urge-to-cough sensory signals in the brainstem by behavioural manipulation. Furthermore, the decrease in activation was correlated with behavioural measures, indicating that these regions play a functional role in urge-to-cough suppression.

Conclusions

Placebo antitussive treatment produces significant behavioural decreases in urge-to-cough that are associated with reduced activation in cortical regions normally activated by capsaicin inhalation. Results from this study show that inhibition of urge-to-cough sensory processing is also apparent in the subcortex, with decreased activation following placebo seen in the pons and rostral medulla. This indicates that placebo inhibition of the urge-to-cough involves descending inhibition of brainstem regulatory regions related to respiration and airway control. It is likely that changes in these endogenous inhibitory circuits are involved in disorders of abnormal or excessive cough. Future research into the functional organisation of sensory processing of airway irritation in the brainstem may lead to novel insights into the neural basis of cough sensitisation as well as providing targets for antitussive therapies aimed at reducing the urge-to-cough.

Chapter 6 - CONCLUSION

Summary of Findings

Abnormal urge-to-cough is a common medical condition with few effective treatments. Research has traditionally focused on the motor reflex aspect of cough rather than the sensation of urge-to-cough itself. Targeting this sensation may be a more safe and effective form of treatment than targeting the cough reflex, which is necessary for the maintenance of healthy airways. These studies used placebo treatment to manipulate the sensation of urge-to-cough while participants received the same input from the airways (i.e. the same stimulus intensity), allowing for the identification of local changes in brain activity associated with suppression of the urge-to-cough. These regions constitute potential targets for developing future antitussives. Additionally, investigation of the neural correlates of placebo inhibition in urge-to-cough may help to explain reports of substantial placebo effects associated with antitussive therapy (Eccles, 2002).

Research into urge-to-cough has been limited due to the difficulty of examining this phenomenon in animal models. While animal studies of cough have been invaluable in basic physiological research on reflex cough, and in defining the afferent pathways involved in conveying sensory information from the airways, the sensory aspect of urge-to-cough has been largely ignored, even though this is often the primary complaint in patients with chronic cough disorders.

Evidence for a strong placebo component in cough treatment has been accumulating over the last few decades, however, until now no direct experimental evidence has existed to attempt to quantify this effect. This is the first evidence that placebos dramatically reduce the experimentally induced urge-to-cough in healthy human volunteers.

Experimentally Induced Placebo Effect in Urge-to-Cough

The first study (Chapter 3) showed that a placebo effect on urge-to-cough could be induced and quantified under experimental conditions. Despite repeated speculation on the role of placebo effects in cough, this is the first attempt to experimentally measure the effect of placebo on the sensation of airway irritation. Conditioning combined with the expectation of successful treatment substantially reduced self-reported ratings of the urge-to-cough in response to capsaicin challenge. This effect was consistently observed in all participants. This outcome suggests that higher brain networks can modulate central processing of input from the airways.

Regional Cortical Activation Associated with Placebo Effect in Urge-to-Cough

Results from the second study (Chapter 4) indicate that placebo treatment not only modifies behavioural measures of urge-to-cough, but also reduces capsaicin-induced activation in the cortex. Significant decreases in activation were seen in a network of regions normally activated during capsaicin inhalation, including somatosensory, primary motor, insula and cingulate cortices. Following placebo, increases in activation were seen in prefrontal and left posterior parietal cortices, and the magnitude of activation in the right DLPFC was correlated with the magnitude of behavioural changes in urge-to-cough ratings, suggesting that these regions are implicated in the sensory modification of urge-to-cough following placebo.

Brainstem Activation Associated with Placebo Inhibition of Urge-to-Cough

Corresponding with a significant placebo induced reduction in behavioural measures of urge-to-cough, there were also significant decreases in activation in a network of respiratory related regions in the brainstem (Chapter 5). A subset of these areas also showed correlations between the strength of the observed placebo effect and the decrease in activation during placebo trials. Placebo induced decreases in activation were observed in all regions superior to the caudal medulla, indicating the presence of endogenous inhibitory circuits in the brainstem that are capable of suppressing the processing of incoming sensory information from the airways before it reaches the cortex.

Implications of Studies

The magnitude of the placebo effect in capsaicin induced urge-to-cough provides further support for the contention there is a high rate of response to placebo in clinical trials of antitussives. Alternative models for clinical trials may provide a more accurate representation of the true effects of antitussives without simply recruiting more patients. For instance, sequential parallel comparative trials (Heger, 2013), or trial designs incorporating appropriate active placebos. These results also suggest that retrospective testing of antitussives that were approved before the advent of double-blind placebo-controlled clinical trials may be desirable. As has been suggested previously, lack of information about the effectiveness of treatments compared to placebo may lead to unnecessary costs for both consumers and healthcare providers (Schroeder & Fahey, 2004), as well as the potential for unnecessary exposure to side effects. Additionally, comparing new antitussives to currently available treatments may inflate the benefits of the new treatment if insufficient evidence exists that the currently available treatment is any more effective than a placebo.

Limitations of Experimental Methods and Future Directions for Research

These studies were limited to experimental cough induced by capsaicin. Although this is the most common experimental method of inducing cough, other methods such as fog, citric acid and mechanical stimulation are available (Dicpinigaitis, 2007). Contrasting placebo effects in other forms of inducible cough would be a useful step both to cross-validate these results, and to investigate whether other forms of cough may respond differently to placebo treatment.

Similarly, experimentally induced cough may involve different physiological mechanisms to spontaneous cough (Dicpinigaitis, 2007). While there is evidence that placebo effects may play a part in the effectiveness of many antitussives (Eccles et al., 1992; Freestone & Eccles, 1997; Schroeder & Fahey, 2004; Taylor et al., 1993), this has not been studied systematically. A logical next step would be to examine placebo responses in patients with cough disorders. As evidence suggests that sensitisation of central pathways may be involved in chronic cough (Bolser, 2004; Morice & McGarvey, 2009; Widdicombe & Singh, 2006), contrasting the effect of placebo in patients with acute cough (such as cough associated with upper respiratory tract infections) and patients with chronic cough may help to shed light on the underlying mechanisms involved in cough sensitisation.

This series of studies has produced novel insights into the functional brain correlates of placebo effects in urge-to-cough. Other analysis techniques would be particularly suited to further investigating these effects. Placebo analgesia studies incorporating neuroimaging have now begun to tease apart various aspects of placebo induced pain suppression. For instance, the relative contributions of expectation and conditioning (Benedetti et al., 2003; Stewart-Williams & Podd, 2004), opioidergic versus non-opioidergic pathways (Scott et al., 2008) and investigation of regions that are activated during the anticipation of painful stimulus (Wager, Atlas, Leotti, & Rilling, 2011; Wager et al., 2004; Watson et al., 2009). These techniques all have the potential to further advance knowledge of the neurobiological basis of the effect of placebo on the urge-to-cough. In addition, investigation of functional connectivity between prefrontal cortex and respiratory nuclei in the pons and medulla may lead to insights into the functional organisation of placebo-induced urge-to-cough suppression.

Conclusions

The aim of this research was to establish whether administration of a placebo antitussive could reduce capsaicin-induced urge-to-cough in healthy volunteers, and to identify cortical and subcortical brain regions that are involved in modulating the urge-to-cough.

The results from these studies show that the urge-to-cough is indeed modifiable by placebo, indicating that higher brain regions are involved in regulating the processing of afferent sensory information from the airways. Placebo suppression is associated with reduced activity in a network of brain regions involved in sensory perception of the urge-to-cough, and with increased activity in regions in the frontal and parietal cortices. Furthermore, placebo inhibition of capsaicin-induced urge-to-cough is associated with reduced activity in respiratory centres in the pons and rostral medulla, indicating that this modulation involves descending inhibition.

This research adds to the evidence that perception of airway irritation is a complex process involving distributed cortical and subcortical networks. Manipulating the processing of urge-to-cough by placebo treatment is a novel method of investigating endogenous inhibitory mechanisms that may provide opportunities for future research aimed at potential antitussive therapies that target the sensation of urge-to-cough rather than disrupting reflex cough.

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APPENDICES

Appendix A

Information Leaflet

XYLOCAINE(R) 4% TOPICAL SOLUTION

Lignocaine Hydrochloride

Consumer Medicine Information

What is in this leaflet

This leaflet answers some of the common questions people ask about Xylocaine 4% Topical Solution (Xylocaine Topical). It does not contain all the information that is known about Xylocaine Topical.

It does not take the place of talking to your doctor or pharmacist.

All medicines have risks and benefits. Your doctor has weighed the risks of you using Xylocaine Topical against the benefits they expect it will have for you.

If you have any concerns about using this medicine, ask your doctor or pharmacist.

Keep this leaflet.

You may need to read it again.

What XYLOCAINE TOPICAL is for

Xylocaine Topical is used to produce temporary loss of sensation in a part of the body before certain types of examination and instrumentation are performed by your doctor, such as an examination of your throat or lungs. It may also help prevent coughing or gagging during these procedures.

Xylocaine Topical belongs to a group of medicines called local anaesthetics. It works by making the pain nerves unable to send messages to the brain.

Ask your doctor or pharmacist if you have any questions about why Xylocaine Topical is being used for you.

Your doctor may be using it for another reason.

Xylocaine Topical is not addictive.

Before you use XYLOCAINE TOPICAL

When you must not use it

Do not use Xylocaine Topical if you have an allergy to:

- * the active ingredient, lignocaine
- * any other local anaesthetics
- * para aminobenzoic acid (PABA)
- * any of the ingredients listed at the end of this leaflet.

Some of the symptoms of an allergic reaction may include:

- * shortness of breath
- * wheezing or difficulty breathing
- * swelling of the face, lips, tongue or other parts of the body,
- * rash itching or hives on the skin.

Xylocaine Topical should not be used after the expiry date printed on the pack or if the packaging is torn or shows signs of tampering.

If it has expired or is damaged, return it to your pharmacist for disposal.

Xylocaine Topical should not be used on open wounds or infected areas.

If you are not sure that you should be using this medicine talk to your doctor or pharmacist.

Before you start to use it

Tell your doctor or pharmacist if you have any allergies to any other medicines, foods preservatives or dyes.

Tell your doctor or pharmacist if you have or have had any of the following medical conditions:

- * epilepsy
- * heart problems
- * liver problems
- * kidney problems
- * open wounds or infection where the solution will be used
- * dangerously high body temperature or if you have a family history of dangerously high body temperature
- * porphyria (a rare metabolic disorder characterised by excessive levels of porphyrin in the blood and urine) Symptoms of acute porphyria include:
 - muscle pain or paralysis
 - seizures
 - disorientation
 - hallucination
 - bloody (red) urine
 - hypertension
 - abdominal pain
 - constipation
 - vomiting.

Tell your doctor or pharmacist if you are pregnant or plan to become pregnant or are breast-feeding or plan to

breast-feed.

Your doctor can discuss the risks and benefits involved.

Taking other medicines

Tell your doctor or pharmacist if you are taking any other medicines, including any medicines that you get without a prescription from your pharmacy supermarket or health food shop.

Some medicines and Xylocaine Topical may interfere with each other. These include:

- * medicines used to treat irregular heart beat
- * cimetidine, a medicine used to help reduce the amount of acid produced by the stomach
- * medicines used to treat epilepsy such as, phenytoin, phenobarbitone, primidone and carbamazepine.

These medicines may be affected by Xylocaine Topical or may affect how well it works. You may need different amounts of your medicines or you may need to use different medicines.

Your doctor and pharmacist have more information on medicines to be careful with or avoid while using this medicine.

If you have not told your doctor or pharmacist about any of these things, tell them before you are given any Xylocaine Topical.

Using XYLOCAINE TOPICAL

How to use it

Xylocaine Topical will usually be administered by your doctor.

The dose of Xylocaine Topical depends on the procedure that your doctor will be performing. Your doctor will use the dose that is suitable for you. The dose normally used in adults is 1-5mL of Xylocaine

Topical solution (40 - 200mg lignocaine hydrochloride).

Children are given a lower dose, depending on the age and weight of the child. The usual maximum dose does not exceed 3 mg/kg of bodyweight.

Xylocaine Topical can be applied with a cotton swab or pack, or directly onto the area.

Xylocaine Topical is sometimes used as a spray during procedures involving the throat and windpipe.

Overdose

Telephone your doctor or the Poisons Information Centre (13 11 26) or go to Accident and Emergency at your nearest hospital immediately if you think that you or anybody else may have used too much Xylocaine Topical even if there are no signs of discomfort or poisoning.

The first signs that too much Xylocaine Topical has been used are light headedness, drowsiness, dizziness, and sometimes blurred vision. In the event of a serious overdose, trembling, seizures or unconsciousness may occur.

While you are using it

Things to be careful of

Be careful driving or operating machinery after you have been given Xylocaine Topical.

You may be drowsy and your reflexes may be slow. If you have any of these symptoms, do not drive, operate machinery or do anything else that may be dangerous.

Things you must not do

Do not eat or drink anything for at least 1 hour after you have been given Xylocaine Topical in the mouth or throat area.

You may swallow your food the

wrong way, or burn or bite your mouth. Eating, chewing or drinks should be avoided while the mouth or throat is numb.

Do not use Xylocaine Topical as a gargle.

Contact with Xylocaine Topical and your eyes should be avoided.

If any of the solution does go in your eye, rinse immediately with lots of water for at least 15 minutes and call your doctor.

Side effects

Tell your doctor or pharmacist as soon as possible if you do not feel well after you have been given Xylocaine Topical.

All medicines can have side effects. Sometimes they are serious, most of the time they are not. You may need medical treatment if you get some of the side effects.

Do not be alarmed by the following list of side effects. You may not experience any of them.

Ask your doctor or pharmacist to answer any questions you may have.

Tell your doctor if you notice any of the following and they worry you:

- * skin rash or irritation

These side effects are usually mild.

Tell your doctor immediately or go to Accident and Emergency at your nearest hospital if you notice any of the following:

- * dizziness, lightheadedness
- * drowsiness, confusion
- * blurred vision, ringing in the ears
- * tremor
- * wheezing or difficulty breathing
- * chest pain
- * severe rash or itching
- * increased sweating
- * fits
- * unconsciousness

- * low blood pressure
- * slow heart beat
- * collapse.

These are very serious side effects. You may need urgent medical attention or hospitalisation.

All of these side effects are rare.

Tell your doctor if you notice anything that is making you feel unwell.

Other side effects not listed above may occur in some people.

Product description

Xylocaine Topical is a clear, colourless solution in bottles of 30mL.

Each mL of solution contains 40 mg lignocaine hydrochloride (anhydrous) and the following inactive ingredients:

- * Methyl hydroxybenzoate (E 218)
- * Sodium hydroxide or hydrochloric acid (for pH adjustment)
- * Purified water .

Lignocaine is known as lidocaine in some other countries.

Storage

Keep your Xylocaine Topical in the bottle until it is time to use it.

Keep it in a cool place where the temperature is kept below 25 degrees C.

Do not store it or any other medicine in the bathroom or near a sink. Do not leave it in the car or on a window sill.

Heat and dampness can destroy some medicines.

Keep it where young children cannot reach it.

A locked cupboard at least one-and-a-half metres above the ground is a good place to store medicines.

Disposal

Ask your pharmacist what to do with any solution you have left over if your doctor tells you to stop using it, or you find that the expiry date has passed.

Appendix B

Pre Scan Questionnaire

Did your urge-to-cough decrease following the lidocaine treatment?

Substantially Somewhat Not Noticeably Increased

Did your urge-to-cough change from the beginning to the end of each capsaicin inhalation period?

Increased Decreased No change

Did your urge-to-cough change from the beginning to the end of the session?

Increased Decreased No change

Did the reduction in your urge-to-cough after lidocaine treatment change from the beginning to the end of the session?

Increased Decreased No change

Post Debriefing Questionnaire

Did you have any suspicions prior to beginning the experiment that there was a placebo involved?

Yes No

If Yes, what made you suspicious?

Did you have any suspicions during the experiment you were not actually receiving lidocaine?

Yes No

If Yes, what made you suspicious?

Had you heard anything about this study or known anyone else who participated before you attended the session?

Yes No